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CERTIFICATE

This is to certify that the dissertation work entitled " Comparative histologic and immunohistochemical classification of ampullary carcinoma on endoscopic biopsy and resection specimens" submitted by Dr. Usha Mary Abraham is the work done by her during the period of study in the department of Pathology, PSGIMS & R from June 2009 to April 2012. This work was done under the guidance of Dr.V.Nirmala, Professor and Dr. Suma. B. Pillai, Associate Professor, Department of Pathology, PSGIMS & R.

Dr. V. Nirmala,
Professor,
Department of Pathology,
PSGIMS & R,
Coimbatore-641 004.

Dr.Suma.B. Pillai,
Associate Professor,
Department of Pathology,
PSGIMS & R,
Coimbatore-641 004.

Dr. Alamelu Jayaraman,
Professor & Head of the Department,
Department of Pathology,
PSGIMS & R,
Coimbatore-641 004

Dr.S. Ramalingam,
Principal,
PSGIMS & R,
Coimbatore-641 004

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : psgethics2005@yahoo.co.in

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Dr Y S Sivan
Member - Secretary
Institutional Human Ethics Committee

SECRETARY
PSG IMS & R
COIMBATORE-641 004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

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CONTENTS

INTRODUCTION

Junctions of two different types of epithelia can give rise to tumors which may show features of either of the colliding epithelia or a mixture or intermediate of both. One such junction is the ampulla of Vater, the luminal opening of common channel formed by the common bile duct and the main pancreatic duct of Wirsung within the wall of the duodenum. Here the pancreatic and bile duct epithelia meet the duodenal mucosa at the ampulla[(1)].

Tumors involving the ampulla could be arising primarily in the ampulla or extending from the adjacent sites like duodenum, bile duct and pancreas. Tumors arising at any of these sites can present as an ampullary/ periampullary growth. When a neoplasm is centered primarily in the ampulla with or without periampullary mucosal involvement, it is considered a primary ampullary carcinoma [(2)]. These tumors generally have a better prognosis compared to duodenal and pancreaticobiliary neoplasms secondarily involving the ampulla [(3,4).]

Primary ampullary carcinomas arise from the ampulla and present as exophytic or ulcerated lesions. Based on the microscopic appearance these are divided into intestinal, pancreaticobiliary or mixed types[(3,4.)] Distinguishing

between the two primary types has prognostic implications, as the pancreatiobiliary type ampullary carcinomas are said to have a poorer prognosis compared to the intestinal type [5,6.] Morphological analysis alone may not suffice in this context. The role of immunohistochemistry has therefore been explored by various groups of workers. The cytokeratin expression pattern- CK7 Negative / CK20 Positive in the intestinal type and CK7 Positive / CK20 Negative in pancreaticobiliary phenotype was found to correlate with morphology in some of these studies.[7, 8] However studies by other workers contradicted these observations .[9,10] The purpose of the present study was to determine the role of morphology and Cytokeratin profile in accurate typing of ampullary carcinomas as intestinal and pancreaticobiliary.

AIMS & OBJECTIVES

1. To correlate the morphology of ampullary carcinomas with cytokeratin immunoprofile.

2. To determine whether the histopathological & immunohistochemical subgroups correlate with clinical, radiological and known prognostic factors.

MATERIALS & METHODS

Seventy two cases of ampullary carcinoma were received in the department of pathology, PSGIMS&R over a period of 8 years, from January 2003 to October 2011. Of these, 25 were pancreaticoduodenectomy specimens and 47, endoscopic biopsy specimens. After screening the relevant paraffin sections and blocks, thirty cases were selected out of 72, using the following criteria.

1. Histopathological features were unequivocally those of a pure, invasive adenocarcinoma.
2. The neoplasm involved primarily the ampulla of vater, with or without periampullary extension, as judged by gross examination of the resection specimens and by the endoscopic appearance recorded in the case of small biopsies. Those with an exclusively periampullary pattern of growth were not included in the study.
3. Availability of adequate tissue in the paraffin blocks, for further study.

Hematoxylin and Eosin stained paraffin sections of the selected cases were reviewed and the relevant sections with corresponding blocks isolated.

A proforma including points of patient identification, relevant clinical date and details of pathological findings (appendix 1) was prepared and filled in.

Clinical data included the mode of presentation and endoscopic /per operative findings, obtained from case files and pathology request forms. Access to case files in the medical records section was obtained through permission from concerned authorities.

Pathological examination required recording of gross, microscopic and immunohistochemical findings.

A.GROSS EXAMINATION:

a. Resection specimens: The specimens available were examined and findings recorded. In cases where the specimen had already been discarded, the required details were obtained from the gross description in the histopathology report. Location and pattern of growth i.e ampullary or mixed ampullary/ periampullary , size of the lesion, gross morphology i.e ulcerative, polypoid or stenosing, involvement of head of pancreas, duodenal wall, common bile duct and lymph nodes, were recorded for each of the resection specimens.

b. Endoscopic biopsy samples: Whatever information regarding the pattern of growth, size and gross morphology, that could be obtained from the endoscopic observation, were collected from pathology request forms or patient files and recorded.

A.MICROSCOPIC EXAMINATION:

Architecture of the neoplasm i.e tubular, papillary, mixed etc, cell morphology including cell height and nuclear atypia and nature of the stroma were the salient microscopic features included. An attempt was made to classify each of the tumors as intestinal, pancreaticobiliary or mixed based on their appearance in the routine hematoxylin and eosin stained sections.

B. IMMUNOHISTOCHEMISTRY:

Immunostaining for CK 7 and CK20 was done on sections from all the 30 selected blocks using the technique as described below.[11]

TECHNIQUE:

Two cases of colonic carcinoma and one case of cholangiocarcinoma were chosen as controls for CK 7 & CK 20 respectively. 4µ thick sections from the chosen paraffin blocks were made. They were mounted onto the glass slides and were stained with routine hematoxylin and eosin stain. These slides were reassessed for adequacy. Fresh sections from the blocks were cut again at 5µ

thickness and taken on to a Poly-L-Lysine coated slide. Same procedure was carried out on the control sections too.

Immunohistochemistry for the detection of CK 7 & CK 20 expression was done using the super sensitive polymer – HRP detection system along with appropriate controls. 3, 3'diaminobenzidine tetra hydrochloride (DAB) was used as the chromogen. The procedure followed is described below.

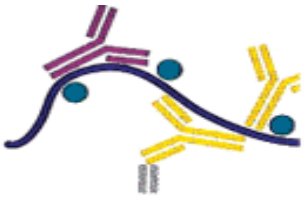
Method: The supersensitive polymer – HRP detection system

Antibody: CK7 & CK 20 (Biogenex)

Principle: Antigens in tissues and cells was detected by a two stage process: the binding of the primary antibody to specific epitopes and the subsequent detection of this binding by a colorimetric reaction using a substrate chromogen.

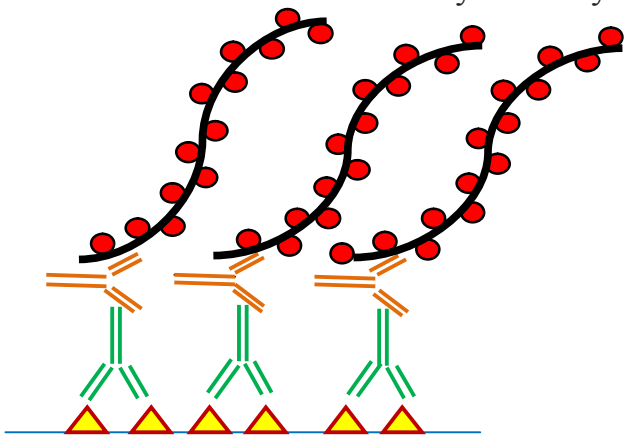
The method is based on the utility of compact dextran polymer to which multiple molecules of the enzyme (Horse radish peroxidase) are attached to each linker secondary antibody (specific for the unconjugated primary antibody). The primary antibody (CK7 & CK 20) for the antigen to be localised is first employed. This is followed by the addition of dextran polymer with multiple conjugated secondary antibodies. Multiple secondary antibodies

react with different antigenic sites on the primary antibody, thereby increasing the signal amplification with the use of a suitable chromogen 3, 3'diaminobenzidine tetra hydrochloride (DAB).



Primary antibody

Secondary antibody



--- Polymerase enzyme

--- Secondary antibody

--- Primary antibody

---- Dextran polymer

---- Tissue antigen

ANTIGEN RETRIEVAL: Various methods are used to expose the antigenic sites (epitopes) that may be unexposed (masked) during routine processing due to formation of cross linking by the action of formalin. The methods to unmask the epitopes include digestion with a variety of proteolytic enzymes, microwave heating, and lastly exposure to the combined effects of heat and pressure in a stainless steel pressure cooker as is said to be more uniform. It can recover almost full antigenicity. In this study, antigen retrieval was carried out using pressure cooker for 10 min, using Citrate Buffer at a pH of 6.0.

REAGENTS:

1. Citrate buffer at pH of 6.0. It was prepared by dissolving Tri sodium citrate (2.94g) in 1000ml of distilled water and 5ml of 1 N Hcl.

2. 3% H_2O_2 in distilled water to block endogenous peroxidase activity.

3. Phosphate buffered saline (PBS) with a molarity of 0.01M and the pH value of 7.6. It was prepared by dissolving the following substances in 1000 ml of distilled water.

Na_2HPO_4 Dibasic sodium phosphate, anhydrate 17.5g

KH_2PO_4 Monobasic potassium phosphate, anhydrous 2.5g

NaCl Sodium chloride 17.0g

4. Blocking reagent- contained casein in PBS with 15mM sodium azide. This was used to blocks non specific protein binding.

5. Primary antibodies against CK 7 & CK 20 mouse monoclonal antibodies supplied in liquid form. (Biogenex).

6. Poly HRP reagent- anti-mouse and anti-rabbit IgG complex linked to Horse radish peroxidase enzyme.

7. DAB (3, 3'Diamino Benzidine tetra hydrochloride) - Chromogen.

It offers great sensitivity as an HRP calorimetric chromogen and provides insoluble permanent coarse brown precipitate.

8. Harris hematoxylin as counter stain.

9. DPX (Distrene dibutyl phthalate Xylene) - Mountant.

PROCEDURE:

1. Sections were cut at 4µ thickness and taken on a egg albumin coated slide.

2. Routine Hematoxylin and eosin stain was done and reassessed.

3. Sections were cut at 5 micron thickness and taken on a Poly-L-Lysine coated slide.

4. Immunohistochemical Staining with CK 7 & CK 20 antigen was done as follows:

a. Slides were dewaxed and dehydrated in graded alcohol.

b. Heat induced antigen retrieval in citrate buffer at pH 6.0 using pressure cooker for 10 minutes

c. Washed in PBS buffer at pH 7.6 for 5 minutes twice

d. Slides were immersed in 0.3% H_2O_2 solution for 20 minutes to block endogenous peroxidase activity.

e. Washed in PBS buffer thrice, each for 5 minutes

f. Slides incubated with blocking solution for 10 minutes to block non-specific protein binding.

g. Washed in PBS buffer thrice.

h. Slides were incubated with CK 7 & CK 20 primary antibody for 1 Hr on the respective sections.

i. Enhancer was applied to the sections for 30 minutes to enhance the signal intensity.

j. Washed in PBS buffer thrice.

k. Slides were incubated with polymer Horse radish peroxidase reagent.

l. Washed in PBS buffer thrice.

m. Diamino Benzidine (DAB) is applied for 7-8 minutes.

n. Washed in PBS buffer thrice.

o. Sections counter stained with Harris hematoxylin for 1 minute.

p. Washed in tap water.

q. Sections cleared in Xylene and mounted with DPX mountant.

The sections were assessed using the light microscope, under low power and high power objectives. Tumors cells were scored positive if there was brown cytoplasmic staining or membranous accentuation in the neoplastic cells. Scoring is done in the well stained area with no interference by nonspecific staining background.

Immunoreactivity was evaluated as follows:

The extent of staining was graded from 0-3 based on the percentage of cells expressing the marker as shown below:

(0) No cells expressing the marker.

(1) < 25% of neoplastic cells expressing the marker.

(2) 25– 50% of neoplastic cells expressing the marker.

(3) > 50% of neoplastic cells expressing the marker.

The intensity of staining was also assessed, though in a subjective manner and graded from 1 + to 3 +. This was done by two individuals independently (UA & VN) and the results were compared.

REVIEW OF LITERATURE

The word ampulla means a flask like dilatation of a tubular structure. The ampulla of Vater is the confluence of the distal common bile duct and the main pancreatic duct in the second portion of the duodenum. In some individuals, the ampulla is a termination of the common bile duct only, as the pancreatic duct enters the duodenum separately next to the ampulla. The ampulla measures 1.5cm in length or lesser, traverses the duodenal wall and open into the duodenal lumen through the major duodenal papilla (papilla of Vater). It is approximately 0.5cm in diameter with mucosal reduplications (valves of Santorrini) and is lined by pancreatobiliary type ductal epithelium with occasional Goblet cells. There is a subtle transition to the intestinal type of epithelium at the duodenal surface of the ampulla. Endocrine and paneth cells are scattered in the non intestinal ampullary mucosa. The lamina propria has occasional lymphocytes, plasma cells and mast cells.[1,3,12,13].

The lymphatics drain into the anterior and posterior pancreaticoduodenal lymph nodes and to the superior and inferior lymph nodes at the head of the pancreas.

Ampullary carcinomas constitute 5% of gastrointestinal carcinomas seen in people aged more than 60 years usually. Clinical symptoms and signs include jaundice, itching, loss of appetite, loss of weight, pale colored stools and others signs of biliary obstruction[14].

Ampullary carcinomas though usually small at the time of diagnosis present with early obstructive symptoms thereby resulting in early detection. Most of these tumors present as a small mass projecting into the duodenal lumen or as periductal thickening or bulging of the papilla. Intrampullary tumors extending into duodenal mucosa can present as an exophytic or ulcerative growth.[12,15]

Spread of these tumors may be invasive or noninvasive. Noninvasive spread includes spread intramucosally to the duodenum as well as to the proximal areas of common bile duct and pancreatic duct. The invasive tumors spread through the musculature into the adjacent duodenal and/or pancreas. The lymph nodes involved are the peripancreatic group. Distant metastasis is usually to the liver, also the peritoneum, lungs and pleura. Vascular and perineural invasion are also known to occur in these tumors.[16,17]

Pathologic staging is difficult due to the complexity of the anatomy of the ampulla of Vater. Ampullary carcinomas have been staged by the AJCC Cancer staging manual 6th edition.[12]

Based on the primary tumor(T) , there are seven stages such as Tx wherein the tumor cannot be assessed, T0 when there is no evidence of the primary tumor, Tis for carcinoma in situ, T1 when the tumor is limited to ampulla of Vater, T2 when the tumor invades duodenal wall, T3 when the tumor invades pancreas and T4 when the tumor invades peripancreatic soft tissues or other adjacent organs or structures.

The problem with this staging is that most invasive carcinomas located at the ampulla of Vater would have invaded the duodenal mucosa by default. So it is difficult to designate a tumor as belonging to stage T1 or T2 based on this criterion. Also , there are rare pancreatic acinar lobules in the wall of the ampulla and therefore invasion of these acini is not considered T3.

Based on the regional lymph node involvement, there are three stages, Nx when the regional lymph nodes cannot be assessed, N0 when there are no regional lymph node metastasis and N1 with regional lymph node metastasis.

Based on distant metastasis there are three stages such as Mx when distant metastasis cannot be assessed, M0 when there are no distant metastasis and M1 when there is distant metastasis.

Pancreaticoduodenectomy is the treatment of choice and the mainstay of therapy. [3,4,18]

The ampullary carcinomas are different from those arising elsewhere in the small intestine in that the ampullary epithelium shows features of both duodenal epithelium and the associated ducts. Accordingly ampullary carcinomas are divided into intestinal and pancreaticobiliary types. Mixed tumors are composed of both types of epithelium. Other less common or rare types are mucinous, signet ring cell, papillary, hepatoid, clear cell, adenosquamous, squamous and medullary carcinomas.[12]

The mucinous adenocarcinomas are the third in frequency of ampullary carcinomas. They are characterized by abundant extracellular mucin and are also referred to as colloid carcinomas.

Signet ring cell adenocarcinomas show a diffuse pattern of individual cells or sheet like or cord like infiltration into the stroma.

Invasive papillary carcinomas may mimic in situ neoplasia because of its exophytic growth, formation of papillae , branching architecture and relatively smooth contours. The stroma is more desmoplastic in this type.

The poorly differentiated adenocarcinomas are made up of poorly differentiated cells growing in sheets.

The medullary carcinomas have pushing borders and show a syncitial growth. Lymphoplasmacytic infiltration of the stroma is also a feature. They are characterized by microsatellite instability and an association with colonic adenocarcinoma. An exceedingly small percentage is constituted by neuroendocrine carcinomas. They occur at a relatively higher proportion in the ampulla. Sarcomatoid carcinomas are very rare.

Intestinal types of ampullary carcinomas consist of elongated, tubular units lined by tall columnar to cuboidal cells with oval nuclei located in the more basal aspects of the cytoplasm and often contain mucin. Pseudostratification of nuclei, solid nests and cribriform areas are also features described in this subtype. The neoplasm bears a resemblance to intestinal carcinomas.[19,20].

The pancreatobiliary carcinomas have simple or branching glands lined by cuboidal to low columnar epithelium in a single layer. They do not exhibit nuclear pseudostratification and the nuclei are rounded with marked pleomorphism. There often is a marked desmoplastic reaction in the stroma in these tumors.[4,5,11,21] The intestinal type carcinomas have a better prognosis when compared to pancreaticobiliary type .[3,4,22,23]

MORPHOLOGY AND IMMUNOHISTOCHEMISTRY CORRELATION

Previous studies have indicated that immunostaining for cytokeratins may help characterize intestinal and pancreatobiliary type of ampullary carcinoma, due to differential expression of cytokeratins, especially CK 7 & CK 20. Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue.[24] Normal hepatobiliary and pancreatic epithelium express cytokeratin7, the small intestinal mucosa expresses cytokeratin 7and Cytokeratin 20 and the colonic epithelium is predominantly cytokeratin 20 positive. [25,26] Gastric mucosa has CK7 and CK 20 variably expressed in different parts & mucosal types.[27] However , it has also been observed that the tumors arising in these locations need not necessarily translate the normal CK pattern.[10]

Jennie et al studied the expression of cytokeratins 7 and 20 in carcinomas of the extrahepatic biliary tract, pancreas and gall bladder. 53 carcinomas were included in the study, of which 8 were of extrahepatic biliary duct, 7 ampullary carcinomas, 11 carcinomas of the gall bladder and 27 carcinomas of the pancreas. The study showed that most tumors of the pancreas and bile duct were CK 7 +ve/ CK 20 –ve tumors. This same pattern of CK 7/20 expresion was also seen in carcinomas of the ampulla, pancreas and majority of gall bladder and bile duct carcinomas. The results failed to support usefulness of CK immunohistochemistry in differentiating pancreatobiliary from intestinal type ampullary carcinoma.[(10)]

Jong Sun Choi et al also studied the expression of cytokeratins 7 and 20 in periampullary carcinomas. 61 resected specimens were included in the study, of which 20 were pancreatic duct adenocarcinomas, 13 distal bile duct carcinomas, 10 duodenal adenocarcinomas and 18 ampulla of Vater adenocarcinomas. $\geq$ 5% tumor cells taking up diffuse staining was considered positive reactivity. The pancreatic ductal adenocarcinomas and the distal bile duct adenocarcinomas were CK 7 +ve/ CK 20 –ve. The duodenal adenocarcinomas and carcinomas of the ampulla were either CK7 +ve/ CK 20 +ve or CK7 +ve/ CK 20 –ve. There was a tendency for increased

CK 20 positivity in less differentiated carcinomas of the ampulla of Vater. It was suggested that CK 7/ CK 20 immunophenotype especially CK 20 expression correlated to some extent with the site of origin of periampullary carcinomas and tumor grade.[28]

Cytokeratins 7, 20 and 17 reactivity were studied in pancreatic and ampulla of Vater adenocarcinomas by Goldstein and Bassi. 64 pancreaticobiliary adenocarcinomas were included in their study. The adenocarcinomas were divided into 3 groups based on the tissue which was infiltrated by the tumor. There groups were ampulla only, pancreas only and ampulla and pancreas. Immunohistochemical staining for cytokeratins 7, 20 and 17 was performed on these groups. The intensity of the stained cells were quantified as weak reactivity, moderate reactivity and strong reactivity. In the ampulla only group, cytokeratins 7 was strongly reactive in all and cytokeratin 20 was positive in 43% of the cases. In the ampulla and pancreas group, strong Cytokeratin 7 reactivity was observed in 91% of the tumors . 23% adenocarcinomas had strong cytokeratin20 reactivity. CK7 +ve/ CK20 –ve pattern was the most common which constituted 55% to 75% of the neoplasms. Though CK 20 expression was more among the ampullary group, the study

could not confirm a useful role for CK profile in the subtyping of these tumors.[9]

Zhou et al were the first to show agreement between the histological classification and immunohistochemical characterization based on cytokeratins. A comparative histologic/ immunohistochemical classification and follow up of carcinoma of ampulla of Vater was done by these authors.. The histologic classification was done according to the histologic criteria published by Albores-Saavedra et al[29]. They found 24 pancreatobiliary, 15 intestinal and 16 other types of carcinomas in a total of 55 cases. They found a high specificity for CK7 for pancreatobiliary type carcinomas and CK20 for intestinal type carcinomas. Most of the tumors correlated by histology and immunohistochemistry. 21 of 24 (87.5%) carcinomas of histologically pancreatobiliary type were of immunohistochemically CK7+ve / CK20-ve. 9 of 15 (60%) intestinal type carcinomas showed the intestinal CK pattern(CK20). 16 carcinomas which were classified as the 'other' type were immunohistochemically divided into 8 pancreatobiliary and 4 intestinal type carcinomas. 4 remained as 'other'. The histologic classification correlated well with the immunohistochemical classification in these tumors.[30]

The expression of cytokeratins 7 and 20 in ampullary carcinomas was studied by Le Pessot et al with an attempt to correlate the immunostaining profile in those tumors. 54 cases of adenocarcinomas were studied retrospectively. The details included were the macroscopic and histological details and immunostaining for cytokeratin 7 and 20. In their study, 26% of the tumors were intestinal, 65% were pancreatobiliary and 9% were of the mixed type. There was a strong correlation between the histological subtype and the CK7/CK20 immunostaining profile.[8]

Cytokeratin 7 & 20 expression were studied for differentiating the tumor subtypes by Roh YH et al. According to these authors, performing immunohistochemical staining was helpful to differentiate between the two types of ampullary carcinomas.[31]

In an attempt to understand the accuracy with which immunohistochemical markers differentiate between pancreatobiliary and intestinal type adenocarcinomas in the pancreatic head, Westgaard et al did a study on 114 resected adenocarcinomas of pancreatobiliary and intestinal types, of which 67 were pancreatobiliary and 47 intestinal. The histopathological features of these tumors were recorded and immunostaining for CK 7 and CK20 was performed. 78% of the tumors morphologically correlated well with

CK7/20 pattern. 58 pancreatobiliary & 30 intestinal tumors were correctly identified by IHC evaluation indicating a moderate agreement between morphology and immunohistochemistry.[32]

MORPHOLOGY-MUCIN TYPE CORRELATION

Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues . Different types of mucins are produced in different parts of the gastrointestinal tract, biliary tract and pancreas. At the ampulla exists a combination of sulphated acid mucins from pancreatic duct, sialomucins from common bile duct and duodenal mucosa and neutral mucins from Brunner's glands.[33] Mucins have been subtyped based on the structure of the core protein into MUC1, MUC2 etc. It has been found that Mucin 1 is expressed in the pancreatobiliary adenocarcinomas and Mucin 2 in the intestinal type.[34,35] Monoclonal antibodies have been developed to these phenotypes. Attempts have been made to correlate ampullary carcinoma subtypes with mucin immunohistochemistry.

Kawabata et al conducted a study on surgically resected specimens from 43 patients with ampullary carcinoma. The tumors were classified based on morphology as intestinal and pancreatobiliary type carcinomas. Tumors with mixed morphology were grouped under intestinal or pancreatobiliary based on

the predominant morphological component. CK 20 and mucin1 expression was studied on these tumors. They found that CK20 had high sensitivity for intestinal type carcinoma (100%) and MUC1 had high sensitivity (94%) for pancreatobiliary type carcinoma.[34] Sessa et al studied 53 resected ampullary adenocarcinomas and analyzed them for MUC1, MUC5 AC, MUC 6, MUC2 using immunohistochemical techniques. The expression of MUC1 and MUC5 AC appeared to be peculiar features of pancreatobiliary adenocarcinoma and a strong production of MUC2 was associated with an intestinal histology.[33]

Other studies have also shown that MUC 2 expression is seen in intestinal type adenocarcinomas and that MUC 1 is associated with pancreatobiliary adenocarcinomas.[34,35]

TUMOR TYPE-SPREAD/PROGNOSIS STUDIES

The clinicopathological findings in the two histologic types of carcinoma of the ampulla of Vater was investigated by Kimura et al.[6] They classified ampullary carcinomas into two types; the intestinal type that resembles the tubular adenocarcinomas of the stomach or colon and the pancreatobiliary type which is characterized by papillary projections with

scant fibrous cores. They did this study on 53 resected specimens of carcinomas of the ampulla of Vater. The pancreatobiliary and intestinal histologic types were found in 38 & 13 cases respectively. Both types of epithelia was found in 11 cases, but they were classified according to the predominant histologic type that was present. Undifferentiated carcinoma was found in 2 cases. They also found that the incidence of lymph node metastasis was higher in cases of pancreatobiliary type than in the intestinal type and that histological infiltration of pancreatic parenchyma was also more frequent in the pancreatobiliary type thereby denoting a worse prognosis for this type of ampullary carcinoma.

Howe et al in their series had 70% of the ampullary cancers with an intestinal morphology which showed a trend toward improved survival as against the pancreatobiliary histologic subtypes (median survival of 59.6 versus 22.5 months)[(5)].

In the study by Le Pessot et al, follow up details of the ampullary carcinomas were documented in addition to the morphology and immunohistochemical staining profile. The five year survival rate was 100% for the intestinal subtype and 40% for the pancreatobiliary type ampullary carcinomas. According to this study, the immunohistochemical staining and

morphological subtype correlated well and that the intestinal subtype had a favorable prognosis.[8]

Roh et al in their series of 34 patients with ampullary carcinoma found that long term survival post resection was significantly greater in intestinal type tumors as against the pancreatobiliary type. These authors had also found statistically effective correlation between morphological subtype and CK7/ CK20 expression.[30]

In a study of 157 patients with ampullary carcinoma correlating tumor histology and survival Jonathan et al found that lymphovascular invasion, perineural invasion, stage and pancreatobiliary subtype predicted survival. Pancreatobiliary type of ampullary carcinoma had worse survival.[9]

In the study by Westgaard[19], histological type of differentiation, tumor origin, pT stage, maximum tumor diameter, resection status, nodal involvement, perineural infiltration, vascular involvement and degree of differentiation were the factors taken into account for the histopathological assessment of the resected specimens. All the tumors were assigned to one of the two histological types. The pancreatobiliary tumors were associated with resection margin involvement, perineural involvement, areas with poor differentiation, lymph node involvement, vessel involvement and tumor size

greater than 25mm and had a poor prognosis . They concluded that histological type significantly discriminates between prognostically poor pancreatobiliary and prognostically good intestinal types of pancreatic head adenocarcinomas.

Georgescu et al explored the relevance of histopathological typing of periampullary tumors for survival in 38 patients. They assessed not only the Histopathological features but also tumor stage , size, degree of differentiation and lymph node involvement. 60.5% of cases were intestinal and 39.5% of the cases were pancreatobiliary in type. The median overall survival was found to be significantly higher in patients with well differentiated intestinal type in T1-T2 stage without lymph node involvement. These authors concluded that intestinal type of periampullary carcinoma has a longer survival but lymph node involvement and degree of differentiation remained significant prognostic factors associated with high mortality[(36)].

Kawabata et al in addition to establishing correlation between tumor morphology, CK20 expression and MUC1 expression , also observed a better prognosis in intestinal type ampullary carcinoma based on pT stage and node metastasis. These authors found that intestinal type carcinoma is slow to progress and carries a lower risk of nodal metastasis when compared to the pancreatobiliary and other unusual histological subtypes[(34)].

The above mentioned studies have been based on correlation between the histomorphologic tumor type and prognosis. Very few studies have been based on molecular characteristics of these carcinomas. One such study is done by Gheza F et al [37]. They studied the differential gene expression between pancreatoduodenal adenocarcinomas and ampullary carcinomas. Among the different genes expressed, it was found that Hepatocyte nuclear factor 4 alpha (HNF4α) was over expressed in ampullary carcinomas(7.61 fold greater) compared to pancreatoduodenal adenocarcinoma. It was also seen that HNF4α expression correlated with its histological subtype, grading , CDX 2 positivity, MUC1 negativity , which are all features of ampullary carcinomas. Multivariate analysis also revealed that HNF4α negativity and lymph node positivity are independent negative predictors of survival. Thereby HNF 4α protein expression is an independent predictor of favorable prognosis in carcinomas of the ampulla of Vater.

Sudo T et al [38] attempted to identify the prognostic factors in patients undergoing pancreatoduodenectomy with lymphadenectomy for ampullary carcinoma. The records of 46 patients with ampullary carcinoma who underwent pancreatoduodenectomy were reviewed. Their overall survival rate was 64%. Univariate analysis revealed that tumor stage, lymph node metastasis and

perineural invasion were significant predictors of poor prognosis. No attempt was made to correlate tumor type with prognosis.

In a review of 36 patients with ampullary carcinoma who underwent pancreatoduodenectomy, Yamaguchi et al [(39)] , studied the prognostic factors. They pointed out that pre operative serum Carcinoembryonic antigen levels, lymphatic permeation, perineural invasion were significant parameters influencing survival post-surgery. Of these , histologic invasion of the venous space was found to be an independent prognostic factor .

A study was done on the pathological factors influencing survival of carcinoma of the ampulla of Vater by Mori et al[(40)]. 24 patients post pancreatoduodenectomy were examined pathohistologically and the prognostic factors were evaluated. Five year survival at stage 1 was 100%, stage 2 was 64.2% and stage 3 was 15%. Only one patient with stage 4 survived for more than 5 years. Localization within the ampulla and lymph node metastasis were significant prognostic indicators. Other factors like shape of the tumor, invasion into veins and lymphatic vessels in the primary lesion and type of local invasion were also indicative of influencing survival. Tumor size, histological type and stromal reaction in the primary lesion did not correlate with survival.

Iacono et al [41] attempted to evaluate the prognostic significance of different clinico-pathological and molecular factors, and to compare survival after standard and extended pancreaticoduodenectomy in ampulla of Vater adenocarcinoma. They have studied 5-10 year survival in 59 patients who underwent pancreatoduodenectomy for ampulla of Vater adenocarcinoma. Surgery alone proved to be curative in patients with only microsatellite instability. But it was inadequate in patients showing chromosome 17p allelic loss. It was suggested that these patients might benefit from adjuvant therapy.

Prognostic molecular factors in ampullary adenocarcinoma could be of significant importance, thus necessitating the need to identify these factors. The possible prognostic significance of cyclooxygenase-2 (COX-2) after surgical resection was thereby studied by Santini.D et al [42]. COX-2 is a rate limiting enzyme which is involved in the conversion of arachidonic acid to H_2 prostoglandin. COX 1 and COX 2 gene have been identified, of which COX 1 is constitutively expressed in many tissues and is involved in many functions such as cytoprotection of the stomach, vasodilation in the kidney and the production of thromboxane by the platelets. COX 2 which has been shown to be involved in carcinogenesis is induced by inflammation or by mitogens, cytokines and growth factors. COX 2 over expression is found in various

histologic types of pancreatic carcinomas and so inhibition of COX 2 could be effective in countering the development of human pancreatic carcinomas. This study examines the possible prognostic significance of COX 2 expression in radically resected ampullary cancer patients. These authors for the first time in the literature , have reported a statistically significant association between high COX 2 expression and poor clinical outcome and can be considered an independent prognostic indicator in ampullary carcinomas. The mechanisms by which over expression of COX 2 is associated with poor prognosis of carcinoma of the ampulla of Vater have not been elucidated. COX 2 expression has been linked to tumor invasion, thereby proving to be a poor prognostic factor for patients with cancer of the ampulla of Vater.

RESULTS

Twenty one out of the thirty cases studied showed correlation between morphology and CK7/CK20 staining pattern. Of these two were intestinal in type, fourteen were pancreaticobiliary type carcinomas and five showed a mixed intestinal pancreaticobiliary morphology as well as immunoprofile (table 1).

MORPHOLOGICAL TYPE	**IMMUNE PROFILE**	**NO. OF CASES**
Intestinal (I)	CK7(-)/CK20(+)	2
Pancreaticobiliary (PB)	CK7(+)/CK20(-)	14
Mixed	CK7(+)/CK20(+)	5
TOTAL		**21**

TABLE 1: MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CORRELATION IN THE SUBTYPES OF AMPULLARY CARCINOMA.

The remaining nine cases showed immunoprofile at variance with morphology. Thus, three cases interpreted as 'intestinal' on morphological grounds turned out to be CK7 positive /CK20 Negative (PB type cytokeratin profile) and two were CK7 positive /CK20 positive (" mixed") cytokeratin profile, another three cases with pancreaticobiliary morphology were CK7 positive /CK20 positive (" mixed"), CK7 negative /CK20 positive (I) and CK7 negative /CK20 negative

(unclassified), respectively. One case had mixed morphological features but showed cytokeratin profile confirming to pancreaticobiliary type carcinoma (CK7 positive /CK20 negative) (table 2).

MORPHOLOGY	**CYTOKERATIN PROFILE**	**NO. OF CASES**
Intestinal	CK7+ / CK20- (PB)	3
Intestinal	CK7+ / CK20+ (M)	2
Pancreaticobiliary	CK7+ / CK20+ (M)	1
Pancreaticobiliary	CK7+ / CK20- (I)	1
Pancreaticobiliary	CK7- / CK20- (-)	1
Mixed	CK7+ / CK20- (PB)	1
	TOTAL	9

TABLE 2: SHOWS THE NINE CASES LACKING MORPHOLOGY IMMUNOHISTOCHEMICAL CORRELATION

AGE AND SEX:

The age of the patient in the present study ranged from 38 to 74 yrs, the overall average being 56yrs. The average age for intestinal type carcinoma was 58yrs, for pancreaticobiliary type 57yrs and for the mixed type 56yrs.

Of the twenty one cases with morphological and immunohistochemical correlation, sixteen were men and five women. No difference in the incidence of tumor subtypes between the sexes was observed, pancreaticobiliary type being the comment in both (M-12/16 and W- 4/5).

CLINICAL PRESENTATION:

All the patients presented with obstructive jaundice. Some complained of loss of weight and abdominal pain and a few presented with recurrent pancreatitis.

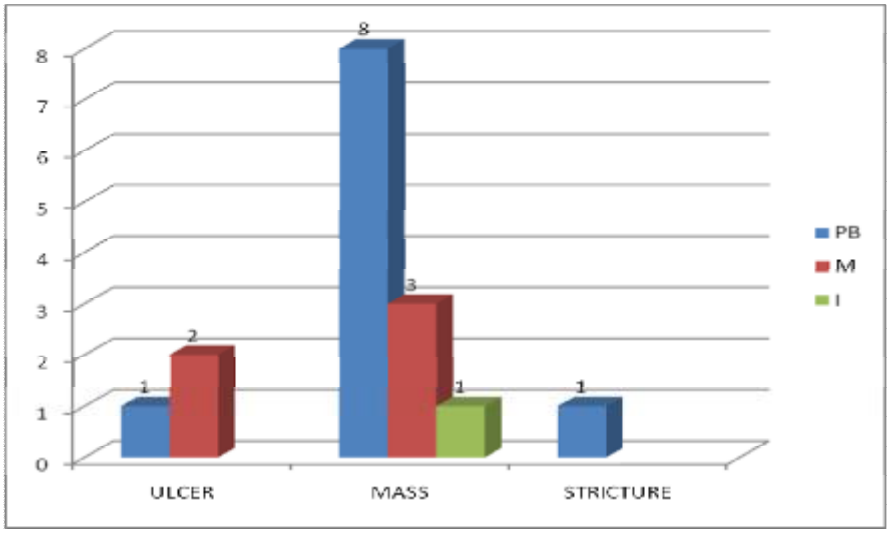
RADIOLOGICAL FINDINGS AND TUMOR TYPE:

Radiological findings were obtained from the hospital records in ten cases. Of these only six had correlation between morphology and cytokeratin pattern. All but one (5 cases) showed dilatation of biliary duct system. One case showed a mass lesion. All the five cases with dilated ducts on radiological examination were of pancreaticobiliary subtype and the one case recorded as “mass lesion” was of mixed type.

ENDOSCOPIC FINDINGS AND TUMOR TYPE:

Endoscopic findings were available in nineteen cases, of which sixteen had morphological-cytokeratin correlation. The lesions had been described as ulcer/ulceroproliferative growth (3 cases), mass /polyp/ampullary prominence (12 cases) and stricture (1 case).

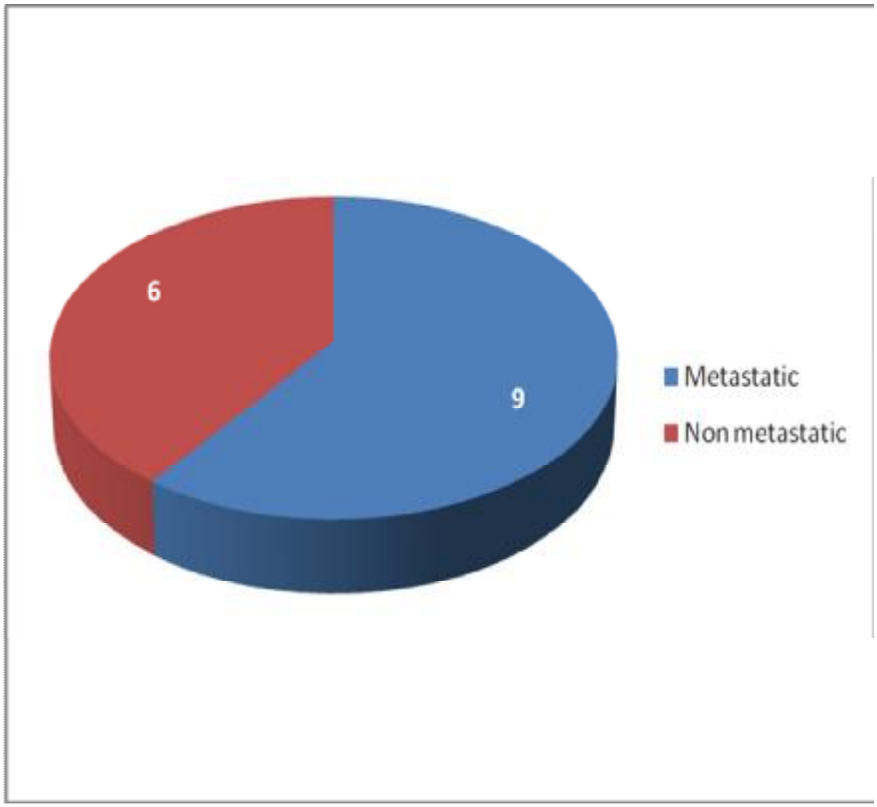
8/10 of the pancreaticobiliary subtype presented as mass lesion/ampullary prominence and one each as ulcer and stricture. Mixed tumors were five in number, of which two were ulcerative and three were mass lesions. The sole intestinal subtype in this group of sixteen also presented as mass lesions. In short there was considerably overlap in the endoscopic findings among the three subtypes (Bar diagram-1).



BAR DIAGRAM 1: DISTRIBUTION OF THE THREE SUBTYPES AMONG ULCER/ MASS LESION/ STRICTURES.

LYMPH NODE METASTASIS AND TUMOR TYPES

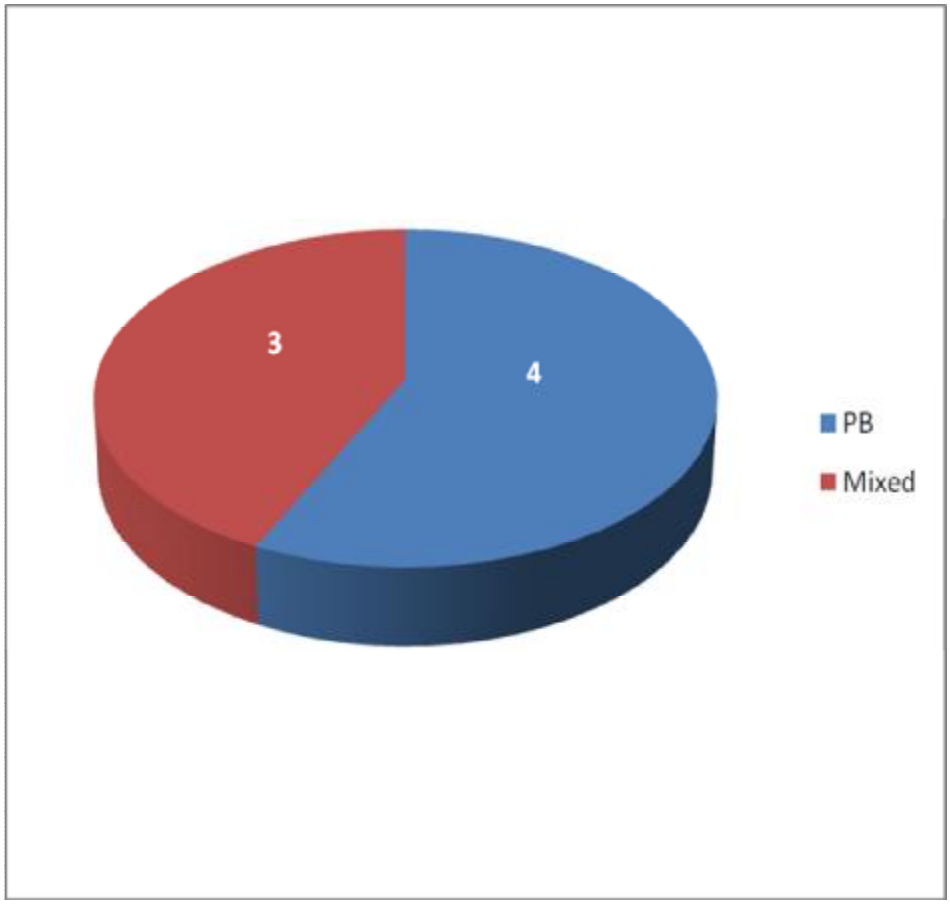
A total of fifteen Whipple's resection specimens were included in the study, of which nine showed metastatic deposits in the lymph nodes(Pie diagram 1). Of these, only seven had morphology correlating with cytokeratin profile, enabling the tumors to be subtyped.



PIE CHART 1- LYMPH NODE METASTASIS IN 9/15 WHIPPLE'S SPECIMENS.

Four out of seven cases with lymph node metastasis were of pancreaticobiliary type and three were mixed carcinomas. Pure intestinal carcinomas did not show

lymph node involvement . The numbers of this subtype however was too small (2/15) to arrive at any definite conclusion.

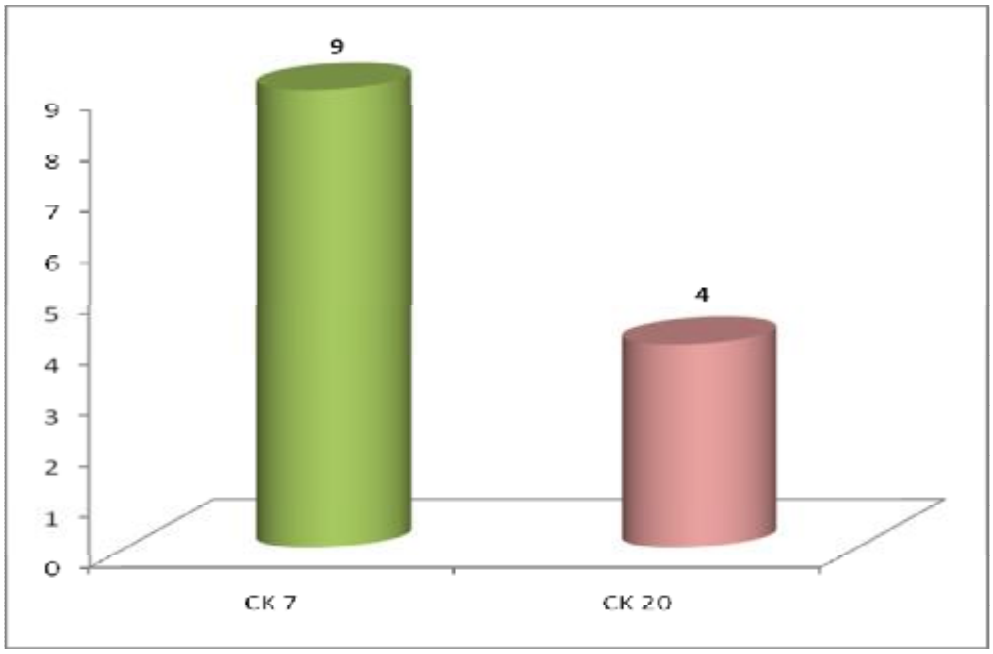


PIE CHART 2- DISTRIBUTION OF PANCREATOBILIARY & MIXED SUBTYPES AMONG 7 TUMORS WITH METASTASIS WHICH HAD MORPHOLOGY-IHC CORRELATION

The two cases with no morphological immunohistochemical correlation, which also showed tumor in lymph nodes, were of pancreaticobiliary (morphology intestinal) and mixed (morphological pancreaticobiliary) by cytokeratin profile . Of the six cases without lymph node metastasis, three had morphological and immunohistochemical correlation. There was one each of intestinal, pancreaticobiliary and mixed subtypes in these three. Of the remaining three, two were mixed and one was intestinal by immunoprofile.

Regardless of morphological subtypes, it was observed that 9/9 tumors(100%) with lymph node metastasis expressed CK7 while only 4 out of these 9 expressed CK20 (44%).(Bar diagram 2).

Of the six cases without metastasis 5(83%) expressed CK20 while only 4 (67%) expressed CK 7



BAR DIAGRAM 2: CK 7 & CK 20 EXPRESSION IN AMPULLARY CARCINOMAS WITH LYMPH NODE METASTASIS.

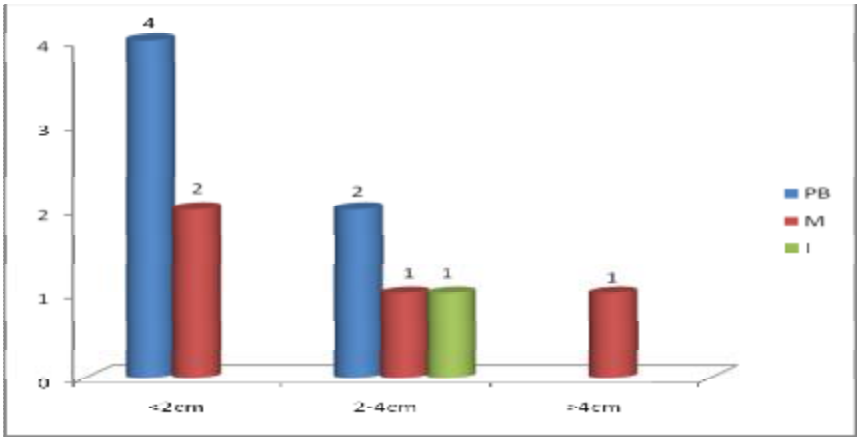
PATHOLOGICAL FINDINGS:

GROSS MORPHOLOGY:

Fifteen of the thirty selected cases were resection specimens. Eleven of these fell within the twenty one cases with histomorphological immunohistochemical correlation.

Tumor size ranged from 0.8x0.5cm to 5.0x3.0cm in the entire group of fifteen resection specimens. Among the eleven lesions mentioned above six measured <2.0cm in the greatest dimension, four measured between 2.0cm and 4.0cm and one >4.0cm across.

Of the six tumors that were < 2.0cm across four were of pancreaticobiliary type (4/6-66%). Of the five tumors measuring > 2.0cm across, three were of mixed or intestinal type (3/5 60%) (Bar diagram 3) . Smaller tumors were predominantly pancreatobiliary, while the larger ones were predominantly mixed and intestinal.



BAR DIAGRAM 3: CORRELATION BETWEEN TUMOR SIZE AND SUBTYPE.

HISTOLOGICAL FEATURES AND CYTOKERATIN PROFILE

Morphological analysis of the hematoxylin and eosin stained sections included assessment of architecture, cell height, nuclear grade and stromal desmoplasia. Based on these features all the thirty neoplasms had been tentatively grouped as pancreaticobiliary (PB), mixed (M) and intestinal (I) types. Now an attempt was made to correlate each of these histological features with CK7 and / or CK20 expression (pie charts 3&4).

ARCHITECTURE:

The main architectural patterns observed were tubular (T), tubular and papillary (TP) and cribriform with solid areas(C.S) in some. Twenty six out of the thirty (26/30) cases expressed CK7. Seven out of these twenty six expressed a tubular pattern (figure 1), eleven of twenty six expresed T-TP pattern and eight of twenty six had C.S pattern.

Eleven out of the thirty cases expressed CK20 (11/30). All the eleven had tubular- papillary (figure 6), cribriform or solid architecture (100%). None showed an exclusively tubular pattern.

ARCHITECTURE	CK7/CK20	NO	TOTAL NO
Tubular	CK7(+)/CK20(-)	7	8
	CK7(-)/CK20(-)	1	
Tubular-papillary	CK7(+)/CK20(-)	8	14
	CK7(+)/CK20(+)	3	
	CK7(-)/CK20(+)	3	
Cribriform- solid	CK7(+)/CK20(+)	5	8
	CK7(+)/CK20(-)	3	
TOTAL 30			

TABLE 3: TUMOR ARCHITECTURE AND CK PROFILE.

Pure tubular pattern is seen only in CK7positive and CK 20 negative tumors. CK20 positive tumors show either mixed tubular-papillary or cribriform- solid architecture.

CELL HEIGHT AND CK EXPRESSION

Twelve of the neoplasms assessed consisted of cuboidal and /or low columnar cells. Eleven of these showed CK7 +/CK20 - immunoprofile and one was CK7 -/ CK20-.

Eighteen tumors had tall columnar cells either exclusively or in combination with cuboidal/low columnar cells types (figures 11&12). Seven of these were CK7 (+)/CK20 (-), eight were CK20 (+)/ CK7 (+) (figures 13&14) and three were CK7 (-)/ CK 20 (+) (figures 9&10).

While CK7 expression showed no significant correlation with cell height all the eleven CK20+ cases had a considerable proportion of tall columnar cells.

NUCLEAR GRADE AND CYTOKERATIN PROFILE.

Ten out of ten cases with +++ nuclear grade(figure 2) show CK7+ expression. Nine of eleven cases with CK20 expression are of low to medium nuclear grade (figure 7).

CK PROFILE	NUCLEAR GRADE		
	+	++	+++
CK7(+)/20(-)	4	6	8
CK7(-)/20(-)	0	1	0
CK7(+)/20(+)	1	5	2
CK7(-)/20(+)	2	1	0
	TOTAL- 20		TOTAL 10

TABLE4: NUCLEAR GRADE AND CK PROFILE.

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DESMOPLASIA AND CYTOKERATIN PROFILE.

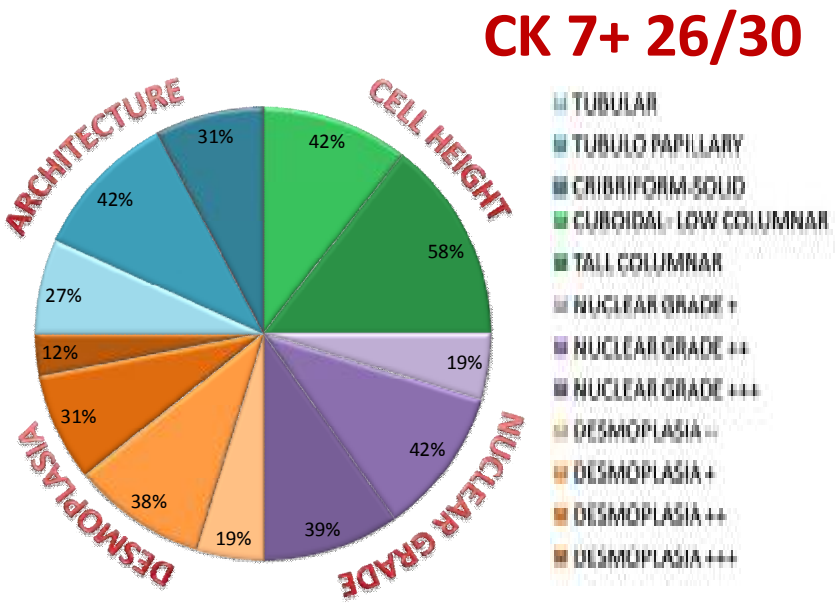
CK7 expression was present in 11/12(92%) tumors with 2 to 3+ desmoplasia (figures 3&4) (92%) and CK 20, in 5/12 (42%) (figure 5).

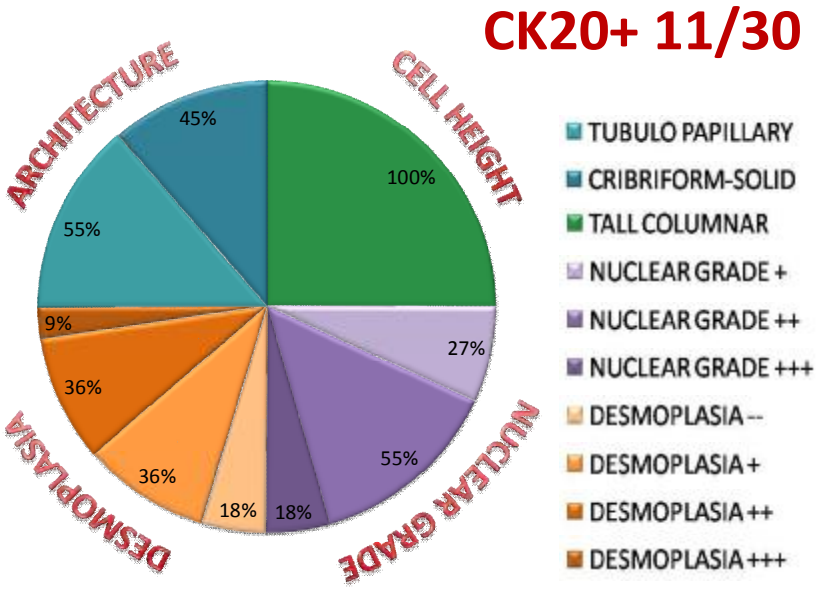
In tumors with 0-1+ desmoplasia (figure 8), 83% expressed CK7 (15/18) and 33%, CK20 (6/18) (figure 9).

CYTOKERATIN PROFILE	DESMOPLASIA				
	+	++	+++	-	
CK7(+)/20(-)	7	5	2	4	
CK7(-)/20(-)	1	0	0	0	
CK7(+)/20(+)	3	3	1	1	
CK7(-)/20(+)	1	1	0	1	
TOTAL	12	9	3	6	30

TABLE 5: DESMOPLASIA AND CYTOKERATIN PROFILE

SUMMARY OF HISTOLOGICAL FEATURES AND CYTOKERATIN EXPRESSION





PIE CHARTS 3 & 4

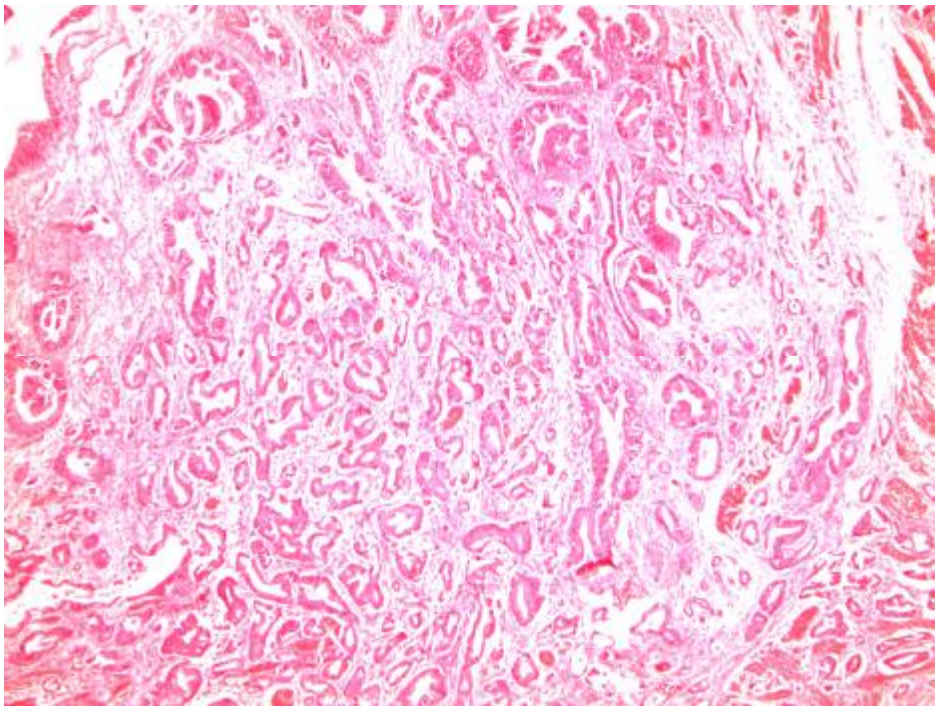


FIGURE 1. PANCREATOBILIARY TYPE CARCINOMA WITH TUBULAR PATTERN(HEMATOXYLIN-EOSIN ,40X)

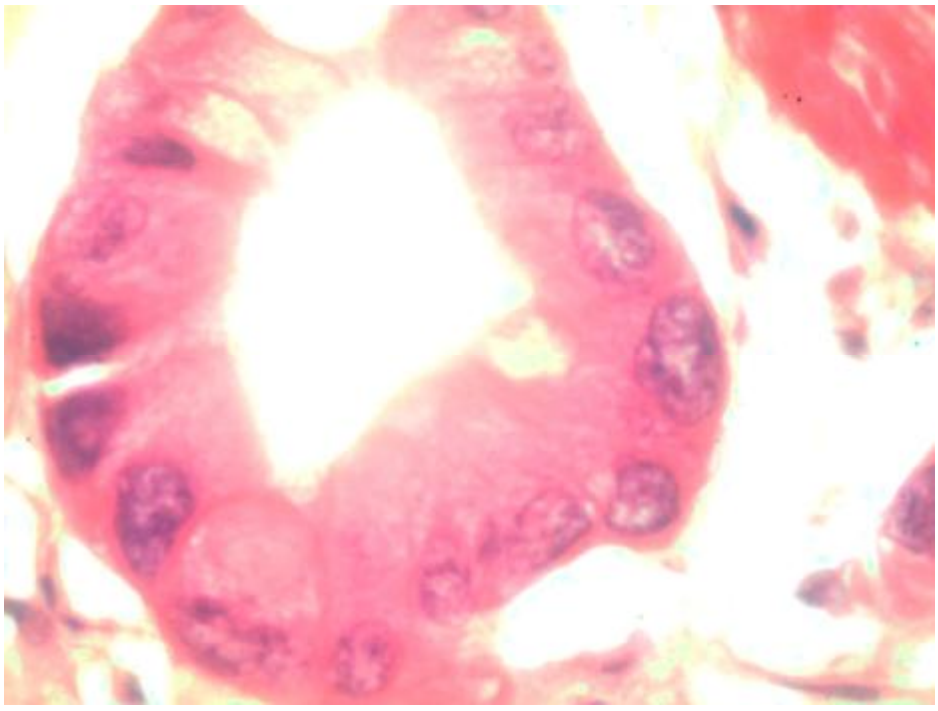


FIGURE 2. PANCREATOBILIARY TYPE CARCINOMA WITH MARKED NUCLEAR ATYPIA (HEMATOXYLIN-EOSIN ,400X)

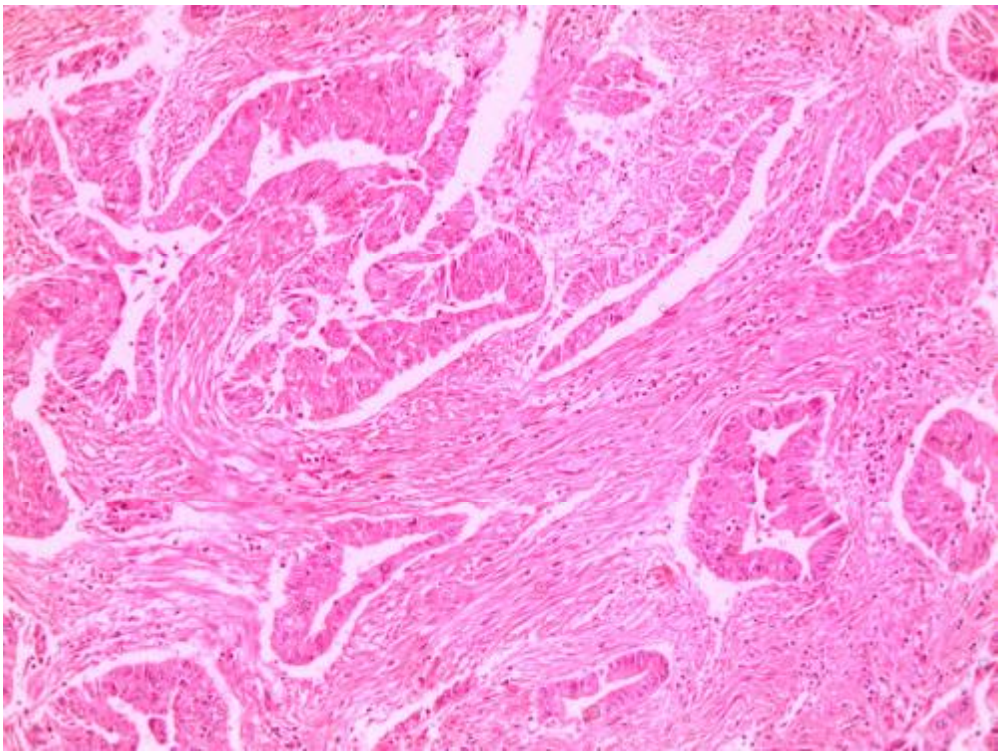


FIGURE 3. PANCREATOBILIARY TYPE CARCINOMA WITH DESMOPLASIA(HEMATOXYLIN-EOSIN ,100X)

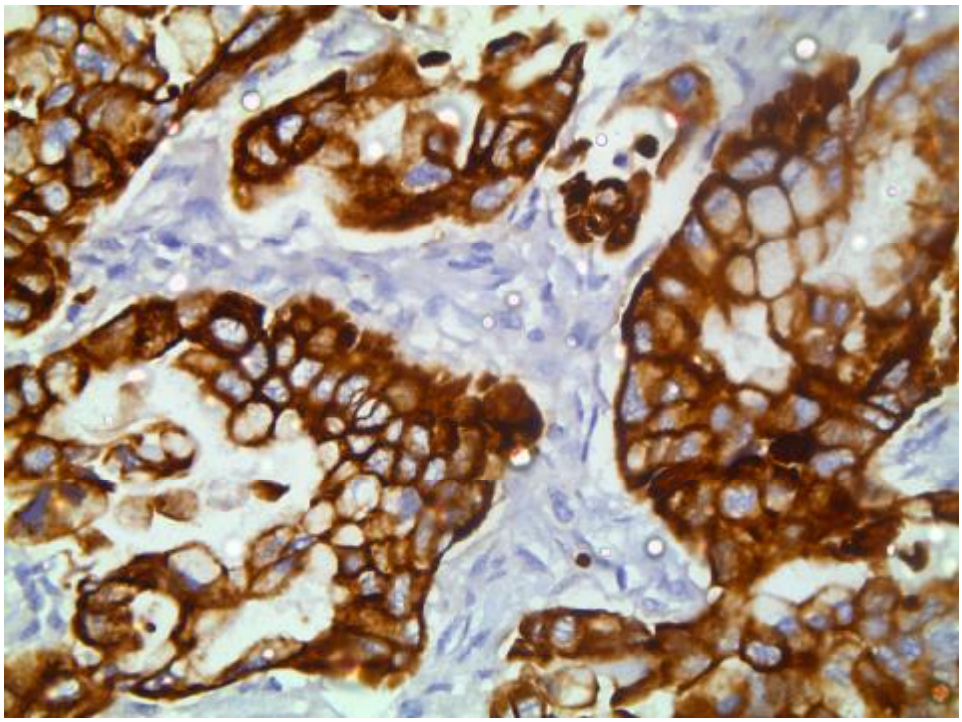


FIGURE 4. PANCREATOBILIAY TYPE CARCINOMA WITH CK 7 POSITIVITY (IMMUNOHISTOCHEMISTRY,400X)

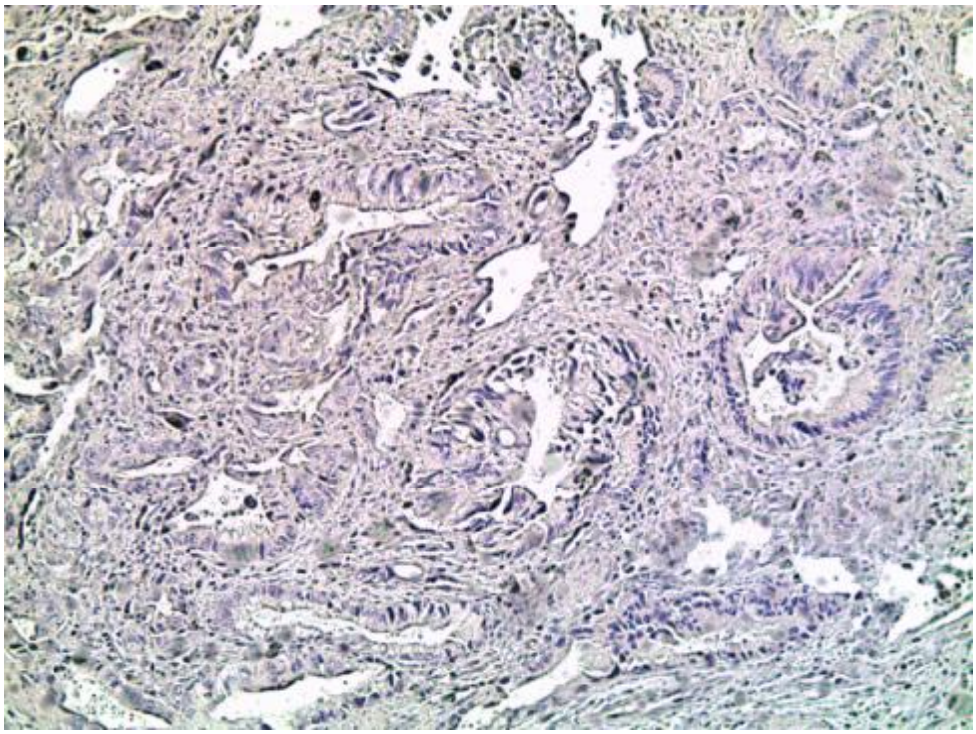


FIGURE 5. PANCREATOBILIARY TYPE CARCINOMA WITH CK 20 NEGATIVITY (IMMUNOHISTOCHEMISTRY,100X)

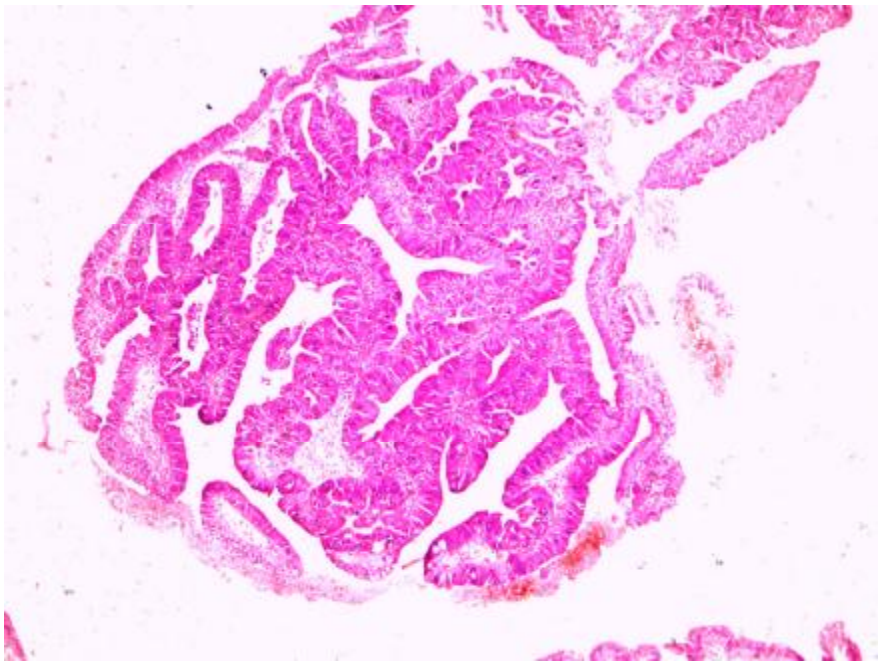


FIGURE 6. INTESTINAL TYPE CARCINOMA WITH TUBULOPAPILLARY PATTERN

(HEMATOXYLIN-EOSIN, 100X)

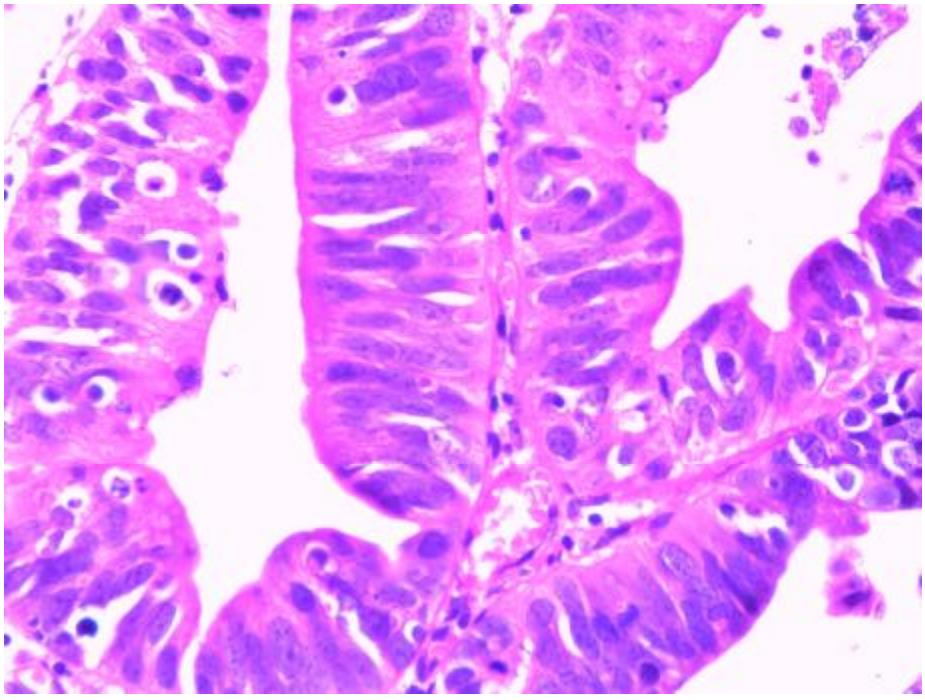


FIGURE 7. INTESTINAL TYPE CARCINOMA WITH MINIMAL NUCLEAR ATYPIA(HEMATOXYLIN-EOSIN ,400X)

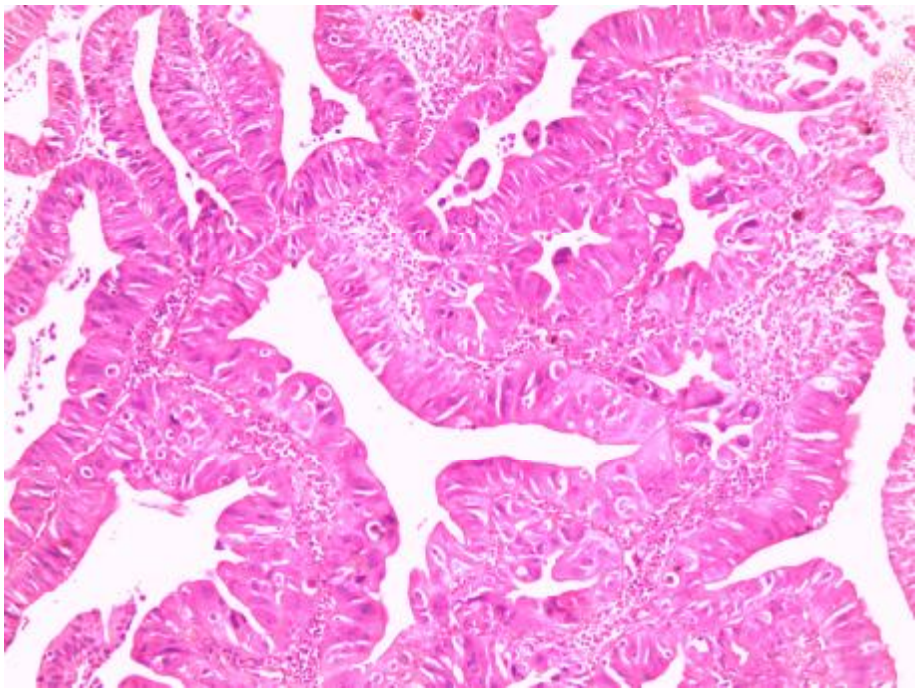


FIGURE 8. INTESTINAL TYPE CARCINOMA WITH ABSENCE OF DESMOPLASIA(HEMATOXYLIN-EOSIN ,400X)

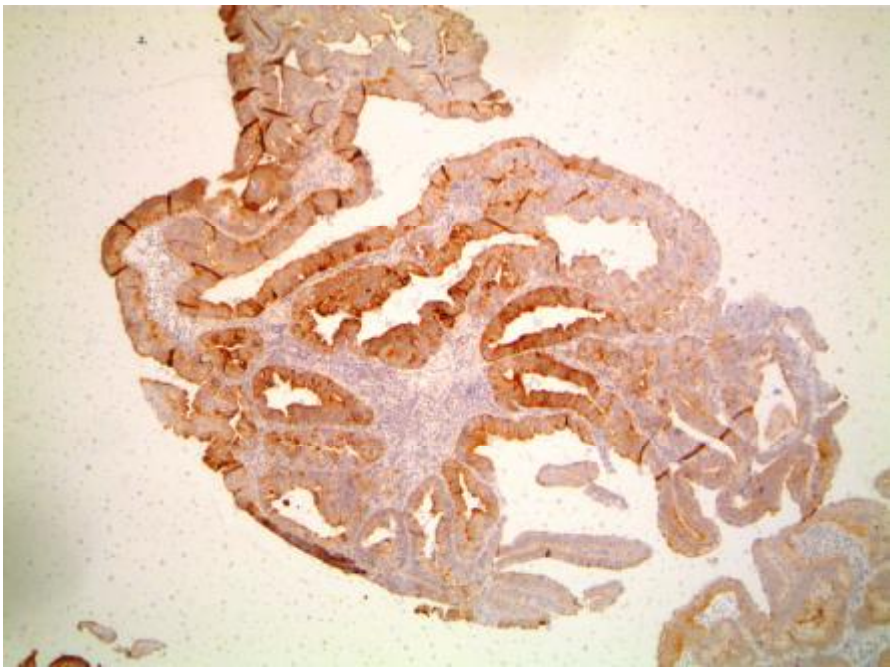


FIGURE 9. INTESTINAL TYPE CARCINOMA WITH CK 20 POSITIVITY(IMMUNOHISTOCHEMISTRY,100X)

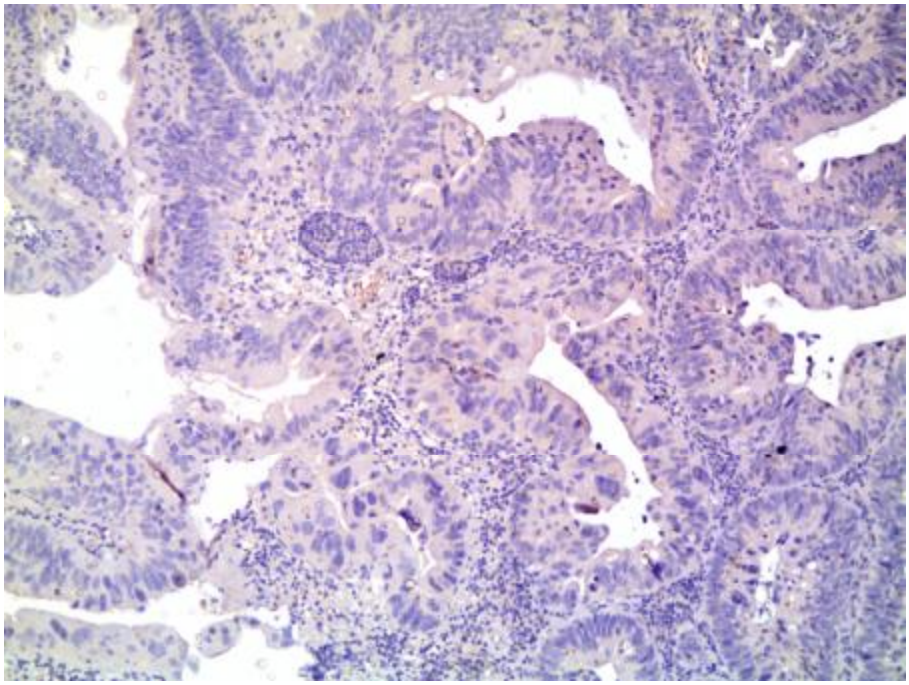


FIGURE 10. INTESTINAL TYPE CARCINOMA WITH CK 7 NEGATIVITY(IMMUNOHISTOCHEMISTRY,100X)

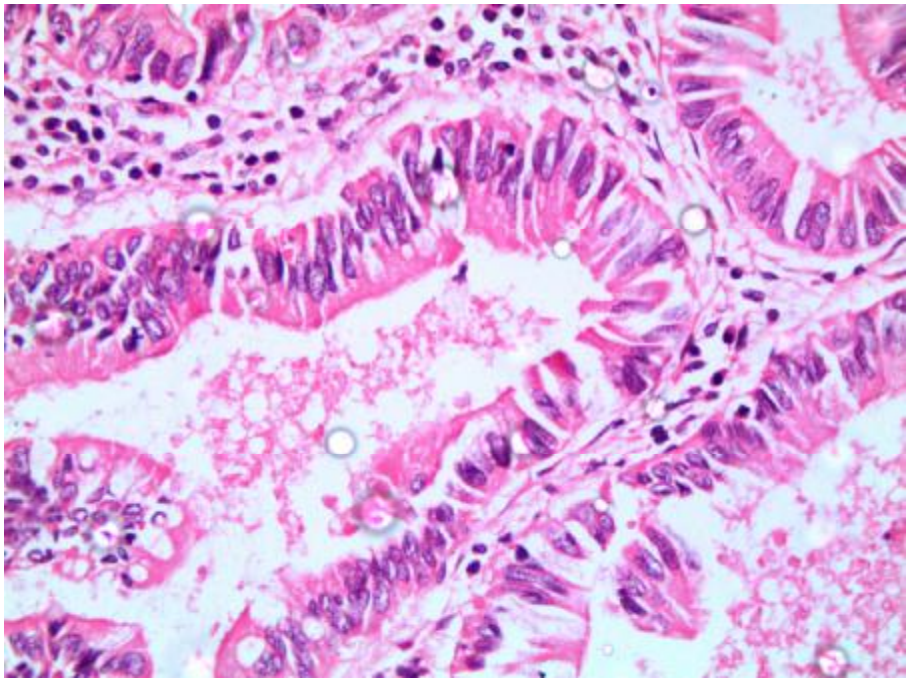


FIGURE 11. TALL COLUMNAR CELLS IN MIXED TYPE CARCINOMA(HEMATOXYLIN-EOSIN ,400X)

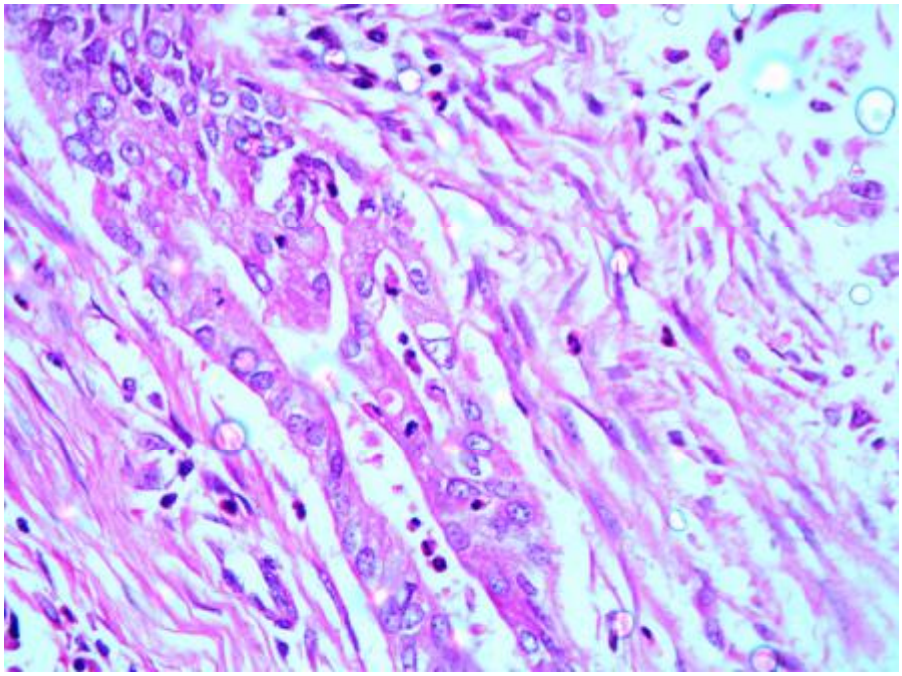


FIGURE 12. CUBOIDAL CELLS IN MIXED TYPE CARCINOMA(HEMATOXYLIN-EOSIN,400X)

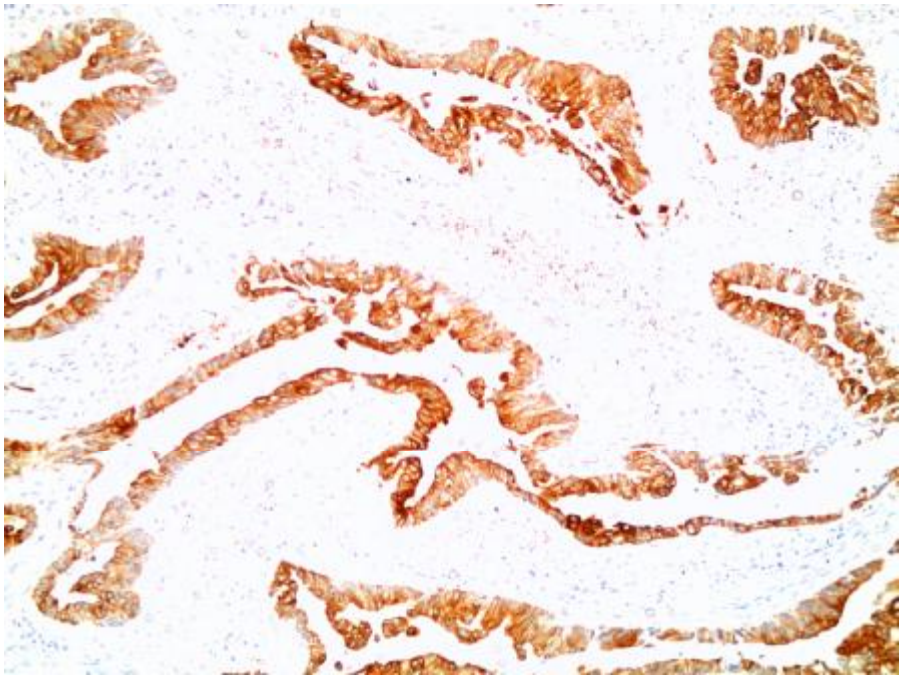


FIGURE 13. CK 7POSITIVITY IN MIXED TYPE CARCINOMA (IMMUNOHISTOCHEMISTRY,100X)

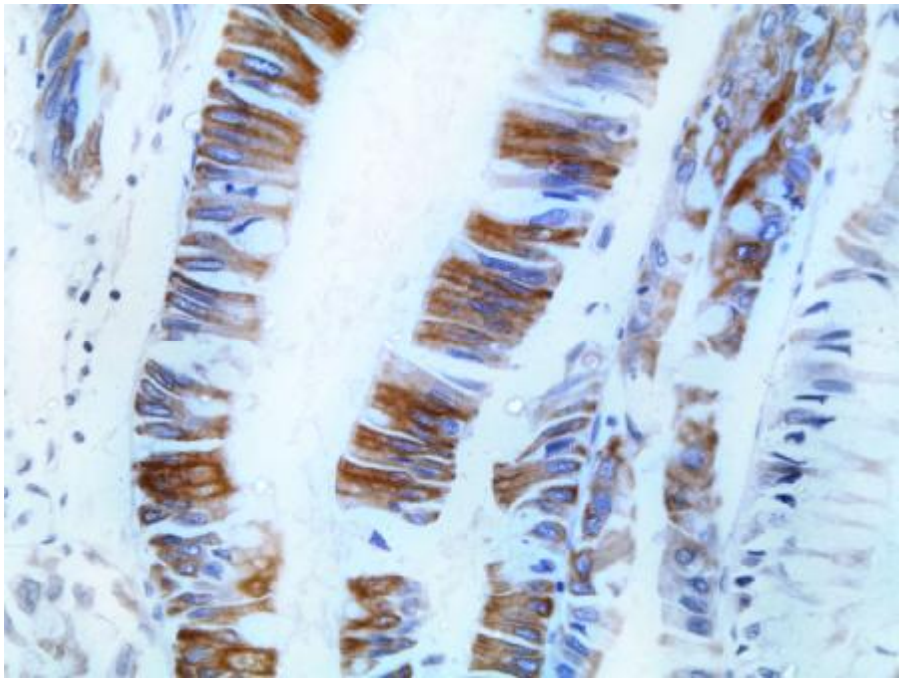


FIGURE 14. CK20 POSITIVITY IN MIXED TYPE CARCINOMA(IMMUNOHISTOCHEMISTRY,400X)

DISCUSSION

Carcinomas of the Ampulla of Vater may arise in the ampulloduodenal part lined by intestinal mucosa or in the deeper part lined by pancreatic or bile duct mucosa. Intestinal and Pancreatobiliary represent the main histological types of ampullary carcinoma. Morphologically the two types resemble their colonic and pancreatic counterparts [(43)]. Moreover intestinal type ampullary carcinomas often possess immunohistochemical marker profile similar to that of colonic carcinomas and the pancreatobiliary type ampullary carcinomas show an immunoprofile resembling pancreatic carcinomas [(44)]. Immunomarkers most useful in this context are cytokeratin 20, cytokeratin 7 and Mucin 2. Thus the intestinal type tumors express "CK 20+, CK7-, MUC 2+" marker profile, while pancreatobiliary type carcinomas express "CK 7+, CK 20-, MUC 2-" profile.

The present study is an attempt to correlate the morphology of ampullary adenocarcinomas with their immunoprofile. The markers used were CK7 and CK 20, both simple cytokeratins with restricted tissue and neoplastic distribution. Cytokeratins are intermediate filaments found in the epithelial cells of all types and are therefore specific markers for an epithelial cell lineage. They have been classified as types 1 through 20. The cytokeratin expression is

frequently organ and tissue specific. The subtypes of cytokeratins expressed depend, in addition to the type of epithelium , on the stage of differentiation and development[45]. Applications of cytokeratin 7 and cytokeratin 20 in combination include study of Barret's mucosa,and assessment of metastatic carcinomas with unknown primary, in addition to typing of ampullary carcinomas.

Cytokeratins 7 and 20 expression could also have a bearing on the prognosis of carcinomas irrespective of subtype. At least in one series, on colorectal carcinomas , cytokeratin 20 expession was found to be more in low grade tumors and cytokeratin 7 expression more in tumors with lymph node metastasis [45]. Other studies have however contradicted these observations [26].

In the present study , 70% of ampullary carcinomas (21/30) showed correlation between morphology and CK7/CK20 profile. Some of the previous studies have also yielded similar results [7,8]. In the series of Zhou et al overall correlation was present in 76% of cases and pancreatobiliary group alone showed a higher percentage of correlation between morphology and cytokeratin profile.

Clinical , radiological and endoscopic data showed considerable overlap in the present study. Lymph node metastasis was found to be present in

pancreatobiliary and mixed subtypes, but not in intestinal type of ampullary carcinoma. However only two cases out of fifteen Whipple's resection specimens were of intestinal type and therefore no definite conclusion could be drawn. But the cytokeratin 7 and cytokeratin 20 expression taken independently did show that 100% of metastatic tumors were CK 7 (9/9) and only 44% (4/9) were CK 20+. Among the cases without metastasis in our series 83% (5/6) expressed CK 20 and only 63% (4/6) expressed CK 7.

This is in agreement with earlier observations of more cytokeratin 7 expression in colorectal adenocarcinomas with lymph node metastasis i.e cytokeratin expression rather than histological type correlated with metastasis [46].

We have in this study, made an attempt to correlate individual histological features like architecture , predominant cell type, nuclear grade and degree of desmoplasia separately, with CK 7 expression and CK 20 expression, also taken separately. It was found that tumors with an exclusively tubular architecture always expressed CK 7 but not CK 20.

With regard to cell height, CK 20 expression was found to have a strong correlation with tall columnar cells in the sense that all CK 20 + tumors had a tall columnar cell component.

Distribution of the different nuclear grades did not differ between cytokeratin 7+ and CK 20+ tumors. But , 100 % of high nuclear grade (+++) neoplasms expressed cytokeratin 7 and 20 % expressed cytokeratin 20. While this would suggest a correlation between CK 7 expression and high grade, one has to bear in mind that majority of tumors in the present series were either pancreatobiliary or mixed in type, and were CK 7+.

Observations on desmoplasia also followed a similar pattern and the results should be viewed against the fact that majority of the tumors were anyway cytokeratin 7+.

Finally, the significance of our findings can be derived from the fact that accurate histologic typing of ampullary carcinomas will provide predictive information influencing staging and planning of operative procedures. It will also provide a solid basis to analyze pathogenetic mechanisms of these tumors by more advanced methods such as molecular studies. Above all it raises the fundamental question whether ampullary adenocarcinoma is to be considered an entity with its own staging system, or

is to be clubbed under other intestinal and pancreatic carcinomas. The number of cases studied is small. The three subtypes were not represented equally. In spite of these drawbacks the following conclusions can be drawn from the present study.

SUMMARY & CONCLUSIONS

1) Tumor morphology of ampullary adenocarcinomas correlates to an extent with cytokeratin immunoprofile, and morphological- immunohistochemical subtyping is possible in 70% of cases.

2) Frequency of lymph node metastasis is more in CK 7+ tumors as against CK 20+ tumors.

3) Among the microscopic features, an exclusively tubular architecture is associated with cytokeratin 7 expression.Tall columnar cell component is unequivocally related to CK 20 expression.

4) It may further be concluded that correlation between some of the individual microscopic features like architecture and cell height with independent cytokeratin7 and cytokeratin 20 expression is greater than that between intestinal / pancreatobiliary morphological types and combined cytokeratin 7/ cytokeratin 20 profile.

PROFORMA

SERIAL NO: BIOPSY NO:

NAME: OP/IP NO:

AGE/SEX:

CLINICAL DETAILS:

RADIOLOGICAL FINDINGS:

ENDOSCOPIC FINDINGS:

PATHOLOGY:

GROSS:

A. TYPE OF SPECIMEN: SURGICAL/ENDOSCOPIC

B. LOCATION:

INTRAAMPULLARY/PERIAMPULLARY

DUODENUM

PANCREAS

CBD

UNCERTAIN

C. SIZE (GREATEST DIMENSION)

MICROSCOPY:

A. LOCATION:

AMPULLARY/PERIAMPULLARY

DUODENUM

PANCREAS

CBD

UNCERTAIN

B. ARCHITECTURE: TUBULAR/PAPILLARY/CRIBRIFORM/MIXED

C. CELL TYPE: TALL COLUMNAR/LOW COLUMNAR/CUBOIDAL

D. MULTILAYERING: +/-

E. NUCLEAR ATYPIA: +/++/+++

F. STROMA:

G. ADJACENT DUODENAL MUCOSA:

H. ADJACENT DUCT MUCOSA:

I. ADJACENT PANCREATIC DUCT MUCOSA:

EXTENT OF INVOLVEMENT:	GROSS	MICROSCOPY
AMPULLA DUODENUM PANCREAS CBD PANCREATIC DUCT LYMPH NODE MARGINS DYSPLASIA/INVASIVECA		

DIAGNOSIS:

IMMUNOHISTOCHEMISTRY

MARKER	EXTENT	INTENSITY

BIBLIOGRAPHY

1. Frierson H F. The Gross Anatomy and Histology of the Gallbladder, Extrahepatic Bile Ducts, Vaterian System, and Minor Papilla. Am J Surg Pathol 13(2); 146-162, 1989.

2. Rosai J. Pancreas and ampullary region. In: Rosai and Ackerman's Surgical Pathology. Ninth edition. Elsevier, St. Louis, Missouri: 2004.1092-95.

3. Cecilia M. Preiser F, Noffsinger A E, Stemmerman G E, Lantz P E, Isaacson P G. Epithelial tumors of the small intestine. In: GI Pathology An Atlas and Text. Third edition. Philadelphia, Baltimore, New York, London, Beunos Aires, Hong Kong, Sydney, Tokyo : Wolters Kluwer , Lippincott Williams & Wilkins; 2008.482-85.

4. Yantiss R K, Antonioli D A. Polyps of the small intestine. In: Goldblum R R, Odze R R. Surgical Pathology of the GI tract, Liver, Biliary tract, and Pancreas. Second edition. Philadelphia,PA:Elsevier Inc; 2009. 465-6, 586-9.

5. Howe JR, Klimstra DS, Moccia RD, Conlon KC, Brennan MF. Factors predictive of survival in ampullary carcinoma. Ann Surg. 1998 Jul;228(1):87-94.

6. Kimura W, Futakawa N, Yamagata S, Wada Y, Kuroda A, Muto T, Esaki Y. Different clinicopathologic findings in two histologic types of carcinoma of papilla of Vater. Jpn J Cancer Res. 1994 Feb;85(2):161-6.

7. Zhou H, Schaefer N, Wolff M, Fischer HP. Carcinoma of the ampulla of Vater: comparative histologic/immunohistochemical classification and follow-up. Am J Surg Pathol. 2004 Jul;28(7):875-82.

8. Le Pessot F, Ranty ML, Hellot MF, Lemoine F, Ténière P, Testart J, Métayer J. Cytokeratins 7 and 20 immunohistochemistry in ampullary carcinomas. Ann Pathol. 2004 Sep;24(4):312-8.

9. Goldstein N S and Bassi D. Cytokeratins 7, 17, and 20 reactivity in Pancreatic and Ampulla of Vater Adenocarcinomas. Am J Clin Pathol 2001; 115:695-702.

10. Duval J V, Savas L, Banner B F. Expression of cytokeratins 7 and 20 in carcinomas of the Extrahepatic Biliary Tract, Pancreas, and Gallbladder. Arch Pathol Lab Med- Vol 124, August 2000.

11. Jackson P and Blythe D. Immunohistochemical techniques. In : John.D.Bancroft, Marilyn Gamble. Theory and Practice of Histological techniques. Sixth edition. Philadelphia, PA, USA: Elsevier;2008. 433-41.

12. Adsay N V. Gall Bladder, Extrahepatic Biliary Tree, and Ampulla. In: Mills S E, CarterD, Greenson J K, Reuter V E, Stoler M H. Sternberg's Diagnostic Surgical Pathology. Fifth edition.Philadelphia: Lippincott Williams & Wilkins; 2010.1636-43.

13. Schirmacher P and Büchler M W. Ampullary adenocarcinoma – differentiation matters. *BMC Cancer* 2008; 8:251.

14. Chan M, and Adler D G. Ampullary cancer: review and clinical update. Commun Oncol 2010;7:61–66.

15. Kim JH, Kim MJ, Chung JJ, Lee WJ, Yoro HS, Lee JT.Differential Diagnosis of Periampullary Carcinomas at MR Imaging. Radiographics. 2002 Nov-Dec;22(6):1335-52.

16. Westgaard A, Tafjord S, Farstad I N et al. Resectable adenocarcinomas in the pancreatic head: the retroperitoneal resection margin is an independent prognostic factor. *BMC Cancer* 2008; 8:5

17. Stiff M G, Alabraba E, Tan YM et al. Assessment of survival advantage in ampullary carcinoma in relation to tumour biology and morphology. Eur J Surg Oncol. 2009 Jul;35(7):746-50.

18. Michelassi F, Erroi F, Dawson P J et al. Experience with 647 consecutive tumors of the duodenum, ampulla, head of the pancreas, and distal common bile duct. Ann Surg. 1989 October; 210(4): 544–556.

19. Westgaard A, Tafjord S, Farstad I N et al. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMC Cancer 2008, 8:170.

20. Carter JT, Grenert JP, Rubenstein L, Stewart L, Way LW. Tumors of the ampulla of vater: histopathologic classification and predictors of survival. J Am Coll Surg. 2008 Aug;207(2):210-8.

21. Goldstein NS and Silverman J.F. Immunohistochemistry of the gastrointestinal tract, Pancreas, Bile ducts, Gall bladder and Liver. In: Diagnostic Immunohistochemistry. Philadelphia, Pennsylvania: Churchill Livingstone; 2002. 333-64.

22. Chen ZM, Wang HL. Alteration of cytokeratin 7 and cytokeratin 20 expression profile is uniquely associated with tumorigenesis of primary adenocarcinoma of the small intestine. Am J Surg Pathol. 2004 Oct;28(10):1352-9.

23. Tot T. Cytokeratins 20 and 7 as biomarkers: usefulness in discriminating primary from metastatic adenocarcinoma.Eur J Cancer. 2002 Apr;38(6):758-63.

24. Moll R, Löwe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol. 1992 Feb;140(2):427-47.

25. Jovanovic I, Tzardi M, Mouzas I A et al. Changing pattern of cytokeratin 7 and 20 expression from normal epithelium to intestinal metaplasia of the gastric mucosa and gastroesophageal junction. Histol Histopathol (2002) 17: 445-454.

26. Choi JS, Kim NR, Ahn GH, Park CK. Expression of Cytokeratin 7 and 20 in Periampullary Carcinomas. Korean J Pathol. 2000 Jan;34(1):34-38.

27. Saavedra A J, Schwartz AM, Batich K, Henson DE. Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. J Surg Oncol. 2009 Dec 1;100(7):598-605.

28. Roh YH, Kim YH, Lee HW et al. The clinicopathologic and immunohistochemical characteristics of ampulla of Vater carcinoma: the intestinal type is associated with a better prognosis. Hepatogastroenterology. 2007 Sep;54(78):1641-4

29. Westgaard A, Schjølberg A R,Cvancarova M, Eide T J, Clausen O P F,and Gladhaug I P.: Differentiation markers in pancreatic head adenocarcinomas: MUC1 and MUC4 expression indicates poor prognosis in pancreatobiliary differentiated tumours. Histopathology. 2009 February; 54(3): 337–347.

30. Stefan H; Alain P C. Ampullary cancer. Current Opinion in Gastroenterology: 2010 May; 26 (3):280–285.

31. Chu P G, Schwartz R E, Lau S K, Yen Y, Weiss L M. Immunohistochemical staining in the diagnosis of pancreatobiliary and ampulla of Vater adenocarcinoma. Application of CDX2, CK 17, MUC1 and MUC 2. Am J Surg Pathol 2005;29: 359-67.

32. Kawabata Y, Tanaka T, Nishisaka T, Inao T, Nishi T, Yano S. Cytokeratin 20 (CK20) and apomucin 1 (MUC1) expression in ampullary carcinoma: Correlation with tumor progression and prognosis. Diagn Pathol. 2010 Nov 25;5:75.

33. Sessa F, Furlan D, Zampatti C, Carnevali I, Franzi F, Capella C. Prognostic factors for ampullary adenocarcinomas: tumor stage, tumor histology, tumor location, immunohistochemistry and microsatellite instability. Virchows Arch. 2007 Sep;451(3):649-57.

34. Paulsen FP, Varoga D, Paulsen AR, Corfield A, Tsokos M. Prognostic value of mucins in the classification of ampullary carcinomas. Hum Pathol. 2006 Feb; 37(2):160-7.

35. Moriya T, Kimura W, Hirai I, Takasu N, Mizutani M. Expression of MUC1 and MUC2 in ampullary cancer. Int J Surg Pathol. 2011 Aug;19(4):441-7.

36. Georgescu SO, Neacşu CN, Vintilă D, Popa P, Florea N, Mihailovici MS. The histopathologic type of the periampullary tumors. Is it important for survival?. Chirurgia (Bucur). 2009 Nov-Dec;104(6):697-700.

37. Ehehalt F, Rümmele P, Kersting S et al. Hepatocyte nuclear factor (HNF) 4α expression distinguishes ampullary cancer subtypes and prognosis after resection. Ann Surg. 2011 Aug;254(2):302-10.

38. Sudo T, Murakami Y, Uemura K, Hayashidani Y, Hashimoto Y, Ohge H, Shimamoto F, Sueda T. Prognostic impact of perineural invasion following pancreatoduodenectomy with lymphadenectomy for ampullary carcinoma. Dig Dis Sci. 2008 Aug;53(8):2281-6.

39. Yamaguchi K, Nagai E, Ueki T, Nishihara K, Tamaka M. Carcinoma of the ampulla of Vater. Aust N Z J Surg. 1993 Apr;63(4):256-62.

40. Mori K, Ikei S, Yamane T, Yamaguchi Y, Katsumori T, Shibata Y, Arai T. Pathological factors influencing survival of carcinoma of the ampulla of Vater. Eur J Surg Oncol. 1990 Jun;16(3):183-8.

41. Iacono C, Verlato G, Zamboni G, Scarpa A, Montresor E, Capelli P, Bortolasi L, Serio G. Adenocarcinoma of the ampulla of Vater: T-stage, chromosome 17p

allelic loss, and extended pancreaticoduodenectomy are relevant prognostic factors. J Gastrointest Surg. 2007 May;11(5):578-88.

42. Santini D, Vincenzi B, Tonini G, et al. Cyclooxygenase -2 overexpression is associated with a poor outcome in resected ampullary cancer patients. Clin Cancer 2005; 11: 3784-89.
43. Gheza F, Cerv E, Pulcini E et al. Signet ring cell carcinoma of Ampulla of Vater: Demonstration of pancreaticobiliary origin. Pancreas . 2011; 40:791-3.
44. Fischer HP, Zhou H. Pathogenesis of carcinoma of the papilla of Vater. J Hepatobiliary Pancreat Surg. 2004;11(5):301-9.
45. Painter JT, Clayton NP and Herbert RA. Useful immunohistochemical markers of tumor differentiation. Toxicol. Pathol. 2010; 38: 131-41.
46. Bayrak R, Yeni Dunya S and Haltas H. Ck 7 and CK 20 expression in colorectal adenocarcinomas. Pathol. Res. Pract. 2011; 207:156-60

S.NO	BIOPSY NUMBER	AGE/SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
1	2414/10	48/F	Obstructive jaundice since 6 months	CT shows a small enhancing lesion in D2. Endoscopy shows bulky ampulla with small ulcer.
2	2041/10	58/F	Obstructive jaundice x 3months	Endoscopy shows fleshy ampulla
3	2208/09	44/M	Jaundice – 3 weeks	Endoscopy shows nodular mass
4	1134/10	55/F	Obstructive jaundice	Endoscopy shows prominent ampulla
5	1937/10	59/F	Jaundice- 3 weeks	CT shows a periampullary mass with dilated CBD

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
1	Whipple's specimen	Intraampullary	1x1cm	Nodular mass	Infiltration into pancreas is seen.	1/6
2	Whipple's specimen	Intraampullary	0.8x0.5cm	Nodular mass	Infiltration into wall of duodenum and bile duct	-
3	Whipple's specimen	Periampullary	3x1cm	Nodular mass	Infiltration into wall of duodenum, pancreatic duct and focally into pancreatic parenchyma	-
4	Endoscopic biopsy	Intraampullary	-	-	-	-
5	Whipple's specimen	Periampullary	4x3cm	Ulceroproliferative growth	Infiltration into duodenal wall and focally into pancreas	-

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTRY				Inference in IHC	Correlation
Architecture	**Cell height**	**Nuclear atypia**	**Stromal desmoplasia**		**Extent**	**Intensity**	**Extent**	**Intensity**		
Tubule, trabeculae	Low columnar	+++	+	PB	3	++	-	-	PB	√
Tubules, papillae, solid pattern	Tall columnar	+	-	PB	3	++	-	-	PB	√
Tubules, papillae	Tall columnar	+ to +++	-	Intestinal	3	+++	-	-	PB	×
Tubules	Cuboidal	++	+	PB	3	+++	-	-	PB	√
Tubules, papillae	Tall columnar	+	+	Intestinal	-	-	3	+++	Intestinal	√

S.NO	BIOPSY NUMBER	AGE/SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
6	S1883/07	40/M	Jaundice- 1 month	Endoscopy showed a friable prominent ampulla
7	S707/10	72/M	Jaundice- 2 weeks Known case of tuberculosis	Endoscopy showed a periampullary growth
8	S746/10	57/M	Recurrent pancreatitis, abdominal pain – 6 months	Endoscopy- large ampullary growth
9	S810/10	38/M	Jaundice- 2 weeks	Endoscopy- polypoid, pedunculated mass
10	S1184/10	74/M	Obstructive jaundice, abdominal pain- 1 month	USG- mass in the head of pancreas. Endoscopy- prominent ampulla

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
6	Whipple's specimen	Intra ampullary	2x3cm	Friable, nodular mass	Infiltration into pancreas and duodenal wall	1/4
7	Endoscopic biopsy	Periampullary	-	-	-	-
8	Endoscopic biopsy	Ampullary	1.0cm	Nodular mass	-	-
9	Whipple's specimen	Periampullary	5x3cm	Polypoid pedunculated mass	-	2/8
10	Whipple's specimen	Ampullary	1.9x1.5cm	Ill-circumscribed firm, bulky mass	Infiltration into duodenal wall	-

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTRY				Inference in IHC	Correlation
Architecture	**Cell height**	**Nuclear atypia**	**Stromal desmoplasia**		**Extent**	**Intensity**	**Extent**	**Intensity**		
Tubules, cords and groups	Low columnar	++	+++	PB	3	+++	-	-	PB	√
Tubules, papillae, focal cribriform	Low columnar to tall columnar	+ to ++	+	PB	3	+++	-	-	PB	√
Tubules, papillae	Tall columnar	++	-	Intestinal	-	-	2	+++	Intestinal	√
Tubules, papillae	Low columnar to tall columnar	++	++	Int/PB	3	+++	2	++	Int/PB	√
Tubules, cribriform and solid pattern	Cuboidal to tall columnar	+ to +++	++	Int/PB	3	+++	3	++	Int/PB	√

S.NO	BIOPSY NUMBER	AGE/SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
11	S1444/10	68/M	Loss of weight, jaundice since 1 month	Endoscopy- Friable ulcerated ampulla
12	S188/09	57/M	Jaundice, abdominal pain- 1 month	Endoscopy- Duodenal ulcer with a mass in the ampullary region
13	S1264/07	52/M	Jaundice, loss of weight- 1 month	Endoscopy- Ulcer in ampullary region
14	S2691/08	55/M	Abdominal pain and jaundice- 2 weeks	CBD showed an area of hard stricture with occlusion of lumen
15	S3074/09	50/M	Jaundice, fever, vomiting- 3 months	Endoscopy- Ampullary prominence.

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
11	Endoscopic biopsy	-	-	-	-	-
12	Whipple's specimen	Periampullary	1.5x1cm	Ulcer with raised margins	Infiltration into duodenal wall and pancreatic parenchyma	3/6
13	Whipple's specimen	Periampullary	4x2cm	Ulcer with DL	Infiltration into duodenal wall	3/5
14	Whipple's specimen	Ampullary	1.5cm	Edema of ampullary mucosa. Stricture with occlusion of CBD lumen	Infiltration into wall of duodenum, CBD, pancreas	1/5
15	Whipple's specimen	Ampullary	1.5cm	Nodular projection at ampulla	-	1/1

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTRY				Inference in IHC	Correlation
Architecture	**Cell height**	**Nuclear atypia**	**Stromal desmoplasia**		**Extent**	**Intensity**	**Extent**	**Intensity**		
Tubules	Low columnar	+++	+	PB	3	+++	-	-	PB	√
Tubules, papillae	Tall columnar	++	+	PB /Int	2	++	1	+	PB /Int	√
Tubules, sheets and cords	Low columnar to tall columnar	++	++	PB /Int	3	++	1	+	PB /Int	√
Tubules	Cuboidal to low columnar	+++	++	PB	1	+	-	-	PB	√
Tubules, papillae	Tall columnar	+	++	Intestinal	3	+++	-	-	Intestinal	×

S. NO	BIOPSY NUMBER	AGE/ SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
16	S4966/09	65/M	Obstructive jaundice- 4 months	CT- Dilated CBD and pancreatic duct
17	S2724/09	53/M	Jaundice, vomiting- 15 days	CT- I HBR dilatation mass lesion in periampullary region of CBD
18	S853/09	47/M	Abdominal pain, loss of weight and jaundice- 2 months	USG- Dilated CHD, CBD upto terminal end
19	S1281/08	57/M	Obstructive jaundice- 2 months	ERCP- Periampullary growth
20	S4369	66/M	Itching loss of appetite and loss of weight- 2 months	Endoscopy – Growth in D2, dilated CBD.

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
16	Endoscopic biopsy	Intra ampullary		Ampullary prominence	-	-
17	Endoscopic biopsy	Periampullary		-	-	-
18	Endoscopic biopsy	Periampullary		-	-	-
19	Endoscopic biopsy	Periampullary		-	-	-
20	Whipple's specimen	Periampullary and ampullary		Ulceroproliferative growth	Involve adjacent duodenum pancreatic parenchyma and CBD	5/10

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTY				Inference in IHC	Correlation
Architecture	Cell height	Nuclear atypia	Stromal		CK7		CK20			
					Extent	Intensity	Extent	Intensity		
Tubules, cribriform	Low columnar	+++	++	PB	1	+	-	-	PB	√
Tubules, cribriform	Tall columnar	++	-	Intestinal	1	+	1	+	PB/Intestinal	x
Tubules	Low columnar	+	+	PB	2	++	-	-	PB	√
Tubules, cribriform	Low columnar	+++	+	PB	3	+++	-	-	PB	√
Papillary cribriform	Tall columnar	+++	+	PB	3	+++	1	+	PB/Intestinal	x

S. NO	BIOPSY NUMBER	AGE/ SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
21	S2193/16	60/M	Obstructive jaundice- 4 months	Endoscopy- ampullary growth
22	1640/09	60/M	Itching, Abdominal pain and jaundice- 1 month	USG- mass in pancreas Endoscopy- ampullary prominence
23	558/07	52/M	Obstructive jaundice	CT- dilated main pancreatic duct & CBD upto ampulla. No mass lesion
24	4267/09	70/M	Obstructive jaundice	Endoscopy- friable growth ampullary region
25	4084	51/M	Recurrent pancreatitis- 6 months	Endoscopy- ulceration in the periampullary region

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
21	Endoscopic biopsy	Intra ampullary	-	Ampullary prominence	-	-
22	Endoscopic biopsy	Periampullary	-	-	-	-
23	Endoscopic biopsy	Periampullary	-	-	-	-
24	Endoscopic biopsy	Ampullary	-	-	-	-
25	Whipple's specimen	Periampullary	3x2cm	Ulcered lesion with raised borders	-	-

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTY				Inference	Correlation
Architecture	Cell height	Nuclear atypia	Stromal		CK7		CK20			
					Extent	Intensity	Extent	Intensity		
Tubules, papillae	Low columnar	++	+	PB	2	++	-	-	PB	√
Tubules	Cuboidal to low columnar	++	+	PB	-	-	-	-	Intestinal	×
Tubules, papillae	Tall columnar	+	-	Intestinal	1	+	-	-	PB	×
Tubules, papillae	Low columnar to tall columnar	++	++	PB	3	+++	-	-	PB	√
Tubules, cribriform, papillae	Tall columnar to cuboidal	+	+	Intestinal	1	+	2	++	PB/Intestinal	√

S. NO	BIOPSY NUMBER	AGE/ SEX	CLINICAL DETAILS	RADIOLOGICAL FINDINGS
26	4675/10	56/M	Pancreatitis, cholecystitis – 1 month	Dilated IHBR
27	3826/10	65/M	Obstructive jaundice- 1 month	Moderately dilated intra and extra hepatic biliary channels nad pancreatic duct
28	3995/10	55/M	Obstructive jaundice- 2 weeks	ERCP- periampullary growth
29	680/11	55/M	Obstructive jaundice	Endoscopy- periampullary growth
30	2845/11	50/M	Obstructive jaundice	Endoscopy- periampullary ulceroproliferative growth.

GROSS FINDINGS					EXTENT OF SPREAD	
S. No	**Type of specimen**	**Pattern of growth**	**Size**	**Morphology**	**Local**	**Lymph node involvement**
26	Whipple's specimen	Ampullary	1.8x0.8cm	Ampullary prominence	-	2/8
27	Endoscopic biopsy	Periampullary	-	-	-	-
28	Endoscopic biopsy	Periampullary	-	Periampullary nodular growth	-	-
29	Whipple's specimen	Periampullary	4x2.5cm	Ulceroproliferative growth	-	-
30	Endoscopic biopsy	Periampullary	-	Ulceroproliferative growth	-	-

MICROSCOPIC FINDINGS				Impression	IMMUNOHISTOCHEMISTY				Inference in IHC	Correlation
Architecture	Cell height	Nuclear atypia	Stromal		CK7		CK20			
					Extent	Intensity	Extent	Intensity		
Tubules	Cuboidal to low columnar	+++	++	PB	3	+++	-	-	PB	√
Tubules, cribriform	Low columnar to cuboidal (mucinous)	++	+++	PB	3	+++	-	-	PB	√
Tubules, papillae	Cuboidal to tall columnar	+	-	PB/ Intestinal	3	+++	-	-	PB	×
Tubules, papillae, cribriform	Tall columnar to cuboidal	++	+++	PB/ Intestinal	3 in papillary pattern and negative in tubules	+++	2in papillary to 3 in tubules	++ to +++	PB/ Intestinal	√
Papillae, tubules	Tall columnar	++	++	PB/ Intestinal	-	-	3	++	intestinal	×

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Architecture																														
Tubular - T	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**P**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**T**	**P**
Papillary - P		**P**	**P**		**P**		**P**	**P**	**P**			**P**			**P**						**P**		**P**	**P**			**CR**	**P**	**P**	**T**
Cribriform- CR										**CR**						**CR**	**CR**		**CR**	**CR**					**CR**					
Solid etc - S													**S**																	
Cell height																														
Cuboidal – C				**C**						**C**				**C**								**C**			**C**	**C**	**C**	**C**	**C**	
Cuboidal – Low columnar- LC	**LC**					**LC**	**LC**		**LC**		**LC**		**LC**	**LC**		**LC**		**LC**	**LC**		**LC**	**LC**		**LC**		**LC**	**LC**			
Tall columnar - T		**TC**	**TC**		**TC**		**TC**	**TC**	**TC**	**TC**		**TC**	**TC**		**TC**		**TC**			**TC**			**TC**	**TC**	**TC**			**TC**	**TC**	**TC**
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CK7 (+) / (-)	**3+**	**3+**	**3+**	**3+**	**-**	**3+**	**3+**	**-**	**3+**	**3+**	**3+**	**2+**	**3+**	**+**	**3+**	**+**	**+**	**2+**	**3+**	**3+**	**2+**	**-**	**+**	**3+**	**+**	**3+**	**3+**	**3+**	**3+**	**-**
CK20 (+) / (-)	**-**	**-**	**-**	**-**	**3+**	**-**	**-**	**2+**	**2+**	**2+**	**-**	**+**	**+**	**-**	**-**	**-**	**+**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**2+**	**-**	**-**	**-**	**3+**	**3+**